REPORT ON THE

EXPERT PANEL ON ARSENIC CARCINOGENICITY: REVIEW AND WORKSHOP

Prepared by:

Eastern Research Group, Inc.
110 Hartwell Avenue
Lexington, MA 02173
EPA Contract No. 68-C6-0041

August 1997

National Center for Environmental Assessment
U.S. Environmental Protection Agency
Washington, DC

NOTE

This report was prepared by Eastern Research Group, Inc., an EPA contractor, as a general record of discussion during the expert panel meeting. As requested by EPA, this report captures the main points of scheduled presentations, highlights from the panel discussion, and a summary of comments offered by observers attending the workshop; the report is not a complete record of all details discussed, nor does it embellish, interpret, or enlarge upon matters that were incomplete or unclear. This report will be used by EPA as a basis for additional study and work on a new drinking water standard for arsenic. Except as specifically noted, none of the statements in this report represent analyses or positions of EPA.

TABLE OF CONTENTS

Appendixes

I. EXPERT PANEL MEMBERS

R. Julian Preston, Chair

Dr. Preston is the Senior Scientific Advisor for the Chemical Industry Institute of Toxicology and an Adjunct Professor in the Department of Toxicology at North Carolina State University and Duke University. He received his B.A. with honors in genetics and a Ph.D. in radiation biology. He is an internationally known geneticist with expertise in mutagenesis, particularly cytogenetic defects, and has applied this knowledge to cancer risk assessment. Dr. Preston is a member of several professional societies, including the Environmental Mutagen Society, the American Society of Human Genetics, and the American Association for Cancer Research. Specific to this task, Dr. Preston has expertise in DNA repair and mutagenesis, cancer mechanisms and molecular pathology, and the modeling of biological processes associated with cancer and risk assessment. He is the author of more than 120 publications covering the several areas of his research interests.

H. Vasken Aposhian

Dr. Aposhian is a Professor of Pharmacology and Molecular and Cell Biology at the University of Arizona. He received his B.S. from Brown University and his M.S. and Ph.D. from the University of Rochester. He has over 40 years of experience in toxicology, molecular biology, and pharmacology. His research interests include the mechanisms of arsenic detoxification and intoxication; metal toxicity and the mechanisms of intoxication of lead, mercury, arsenic, and manganese; and DNA and gene delivery systems for mammalian cells and intact animals. He is a member of numerous professional societies and has published hundreds of papers and abstracts. Specific to this task, Dr. Aposhian has conducted numerous studies on the enzymatic methylation of arsenic. He has a high level of expertise in DNA repair and mutagenesis, DNA methylation and gene regulation, arsenic metabolism, and arsenic-induced carcinogenicity. He is a member of the National Research Council's Subcommittee on Arsenic in Drinking Water.

Samuel M. Cohen

Dr. Cohen is Professor and Chairman of the Department of Pathology and Microbiology at the University of Nebraska Medical Center. He is also a Professor at the Eppley Institute for Research in Cancer at the Nebraska Medical Center. He received his B.S. in medical science and his M.D. and Ph.D. in oncology from the University of Wisconsin, Madison. Dr. Cohen has received numerous honors and served on the National Research Council's Committee on Comparative Toxicity of naturally occurring carcinogens. Dr. Cohen has extensive experience in conduction research on mechanisms of chemical carcinogenesis, using primarily the urinary bladder as a model system. He has published abstracts, papers, and books on carcinogenesis, risk assessment, and pathology. Specific to this task, Dr. Cohen provides a high level of expertise in cancer mechanisms and molecular pathology, as well as modeling of biological processes associated with cancer, cancer risk assessment, and arsenic-induced carcinogenicity.

Jean-Pierre Issa

Dr. Issa is an Assistant Professor of Oncology at The Johns Hopkins Oncology Center, in the Tumor Biology Division. He has conducted extensive research on DNA methylation, DNA methyltransferase, regulation of gene expression, and inactivation of tumor-suppressor genes in human cancers. Recently, Dr. Issa has been involved in several studies linking exposure to carcinogens with aberrant DNA methylation in cancer. He has published numerous papers on this topic. Specific to this task, Dr. Issa provides a high level of expertise in DNA methylation, gene regulation, and general molecular mechanisms underlying the development of cancer.

Andres Klein-Szanto

Dr. Klein-Szanto is a Senior Pathologist and the Head of the Experimental Histopathology Department at the Fox Chase Cancer Center. He is also an Adjunct Professor in the Department of Pathology and Cell Biology at Jefferson Medical College, Thomas Jefferson University. He earned his medical degree from the University of Buenos Aires. Previous to his current positions, Dr. Klein-Szanto was a Professor at the University of Texas System M.D. Anderson Cancer Center and at the University of Texas Graduate School of Biomedical Sciences. He serves on numerous national and international committees focusing on carcinogenesis, tumor promotion, pathobiology, molecular carcinogenesis, and mechanisms of toxicity. Specific to this task, Dr. Klein-Szanto provides a high level of expertise in cancer mechanisms and molecular pathology, as well as modeling of biological processes associated with cancer.

Colin Park

Dr. Park received a B.Sc. in mathematics from the University of British Columbia and an M.S. and a Ph.D. in applied statistics from Purdue University. Dr. Park has held several positions at Dow Chemical, including Manager and Associate Scientist in the Research Systems and Statistics Division and Manager of the Issues Management and Biostatistics Department. He is active in the Chemical Manufacturing

Association (CMA) Risk Assessment Work Group. He is also a member of the Risk Assessment Subcommittee of the American Industrial Health Council and has been on the Science Advisory Board of the National Center for Toxicological Research. He has authored many papers, including "Biological Assumptions in the Bioassay of Carcinogenicity," "Mathematical Models in Quantitative Assessment of Carcinogenic Risk," and "Development of a Physiologically-Based Pharmacokinetics Model for Risk Assessment with 1,4-Dioxane." Dr. Park has a high level of expertise in biostatistics, cancer risk assessment, and risk management policy for carcinogens.

Toby Rossman

Dr. Rossman is a Professor at New York University Medical Center in the Department of Environmental Medicine and Director of the Molecular and Genetic Toxicology program. She holds degrees in biology, biochemistry, and microbiology, and her main research expertise is genetic toxicology, especially of heavy metals and arsenic. Dr. Rossman was responsible for research demonstrating that arsenic interferes with DNA repair, and her research also provided early evidence that arsenic can potentiate the mutagenic effects of other mutagenic agents. Specific to this task, Dr. Rossman has high levels of expertise in DNA repair and mutagenesis, DNA methylation and gene regulation, cancer mechanisms, and molecular pathology. Recent work on arsenic toxicology in Dr. Rossman's laboratory includes the demonstration of an arsenite efflux pump and the cloning of two genes that confer arsenite resistance.

II. INTRODUCTION

Although arsenic is classified as a human carcinogen by the U.S. Environmental Protection Agency (EPA) and the International Agency for Research on Cancer (IARC), millions of people in the United States are exposed to this chemical through their drinking water. In 1988, the EPA performed a risk assessment for arsenic based on epidemiological data from a Taiwanese population. For many reasons, however—including the fact that exposures in Taiwan were up to 100-fold higher than those commonly occurring in drinking water in the United States—there is a high degree of uncertainty associated with the 1988 dose-response assessment.

Under a mandate contained in the Safe Drinking Water Act Amendments of 1996, EPA is required to propose a new drinking water standard for arsenic by the year 2000, and the Agency is committed to using all relevant data to help establish this new standard. Since the 1988 assessment, there has been considerable activity on the effects of arsenic and its metabolites. Recognizing that some of this information may be relevant to the mode of action of arsenic in inducing cancer, the Agency assembled a panel of seven experts to consider the current state of knowledge about arsenic-induced carcinogenicity, which met in Washington, D.C., on Wednesday and Thursday, May 21 and 22, 1997. Representatives of industry, academia, EPA, and other interested federal agencies attended the meeting as observers (see Appendix A). The meeting was chaired by Dr. Julian Preston of the Chemical Industry Institute of Toxicology in Research Triangle Park, North Carolina.

In its charge to the panel, EPA asked this group of experts for their opinions on whether the body of available data regarding arsenic's mode of action is sufficient to support the adoption of one response model (i.e., linear versus nonlinear) over the other in extrapolating from the relatively high levels of arsenic exposure in the Taiwanese population to the lower exposure levels the Agency will be addressing in the new drinking water standard. Toward this end, and in the context of the Agency's 1996 Proposed Guidelines for Carcinogenicity Risk Assessment (U.S. EPA, 1996), EPA specifically requested the panel to:

- Examine data on the direct and indirect effects of arsenic and its metabolites on DNA, DNA repair, DNA methylation and regulation, mutagenesis and carcinogenicity;
- Comment on the potential mechanisms and mode of arsenic-induced carcinogenesis, including whether there is clear evidence for a mode of action for arsenic-induced carcinogenicity and, if there is not, what the weight of evidence is favoring one mode of action over others.
- Comment on how much, if any, confidence EPA can place in any particular mode of action.

- Give a weight of evidence supporting the use of a linear or nonlinear response model in extrapolating to low-dose arsenic exposures.

As a record of its deliberations, the Agency requested the expert panel to produce a report summarizing its review of the relevant data and describing the consensus or lack of consensus among panel members regarding its conclusions. In addition, the panel was requested to capture in its report the reasoning used in adopting these conclusions.

III. PRESENTATIONS

To begin the meeting, Dr. Preston asked panel members to introduce themselves and to provide a brief description of how their individual areas of expertise relate to the issues to be addressed at the expert panel meeting. Following these introductions, the panel heard several presentations from EPA designed to establish a context for the panel's discussion. Brief summaries of these presentations are provided below.

Proposed 1996 Safe Drinking Water Act Charge on Arsenic

Mr. James Taft, EPA Office of Water

Mr. Taft began by noting that the purpose of his presentation was to give the panel a sense of the complex regulatory history of arsenic. In 1976, the interim primary drinking water regulation for arsenic was established as 50 μg/L, based on a standard originally developed by the U.S. Public Health Service in the 1940s. In 1980, however, the surface water quality criterion for arsenic was set at 0.018 μg/L, initiating what is now a long-standing disparity between the regulatory limits for arsenic in drinking water and in surface water discharge. The controversy intensified in 1986, when the 50 μg/L level was adopted as a national primary drinking water standard for arsenic.

In the wake of these events, a group of environmental organizations known as the Bull Run Environmental Coalition brought suit against EPA. In the settlement eventually negotiated, EPA agreed to propose a new primary drinking water regulation in 1992 and to finalize this regulation by 1994. For a variety of reasons, the Agency did not meet this deadline, nor was it able to meet a subsequently negotiated target of 1994 for the proposed regulation. Arsenic has, however, been the subject of a series of workshops and other information-gathering activities sponsored by EPA over the past 2 years.

Last August, Congress passed the Safe Drinking Water Act Amendments of 1996, which included very specific requirements for arsenic. In the 1996 Amendments, EPA was directed to devise a program of research that would fill critical gaps in the current understanding of the health risks associated with exposure to low levels of arsenic. Although funding falls short of the authorized level of $2.5 million, EPA has received a $1 million appropriation for use in supporting this research. Based on the results of this research effort, which will be conducted wherever possible through cooperative agreements with existing public and private groups, EPA is mandated by the 1996 Amendments to propose a new national drinking water standard for arsenic by January 1, 2000, and to have a final regulation in place by January 1, 2001.

In view of the long lead-time required to plan, conduct, and analyze the results of such an ambitious research program, EPA has opted to base the proposed regulation on information that is already available in the published literature and in studies that are currently under way. Since the 1996 Amendments also require the Agency to revisit drinking water standards every 6 years, there will be ample opportunity for EPA to reevaluate this standard as the results of longer-term research become available.

Figure 1 is a schematic representation of the Research Plan the Agency has developed to address scientific uncertainties about the health effects of arsenic. In addition to the usual channels for funding both internal and external research, EPA has entered into a joint funding agreement with the American Waterworks Association and the Association of California Water Agencies. A request for applications (RFA) has been issued, and research under this jointly funded program is expected to begin in August 1997. Other activities that are expected to contribute to improved characterization of the human health risks associated with low-level exposure to arsenic include several programs of international research, evaluations of the existing data being conducted by this panel and by the National Academy of Sciences (NAS), and a process that is currently under way to revise the methodology EPA uses to establish Maximum Contaminant Level Goals (MCLG).

Arsenic Research

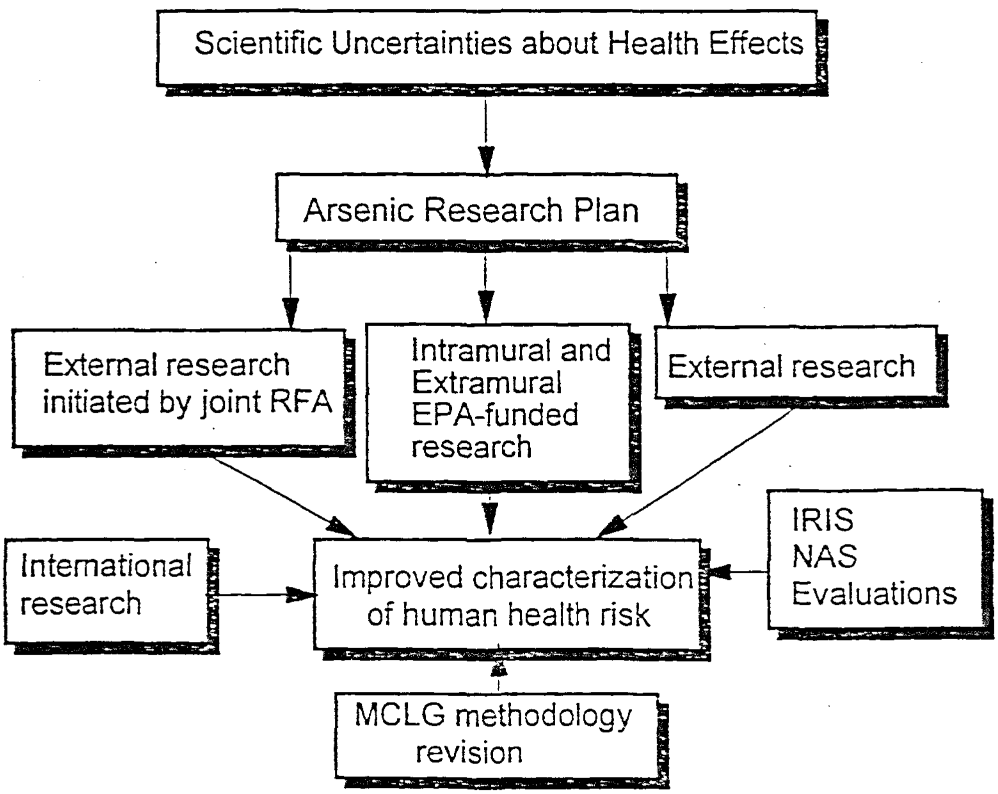


Figure 1. **EPA's approach to addressing scientific uncertainties about the health effects of arsenic**

The Agency's schedule for completion of the various activities that are being undertaken in response to the arsenic mandate is summarized in Figure 2. Because both the writing and review of a new rule are time-consuming processes, the required information will need to be in place well in advance of the statutory deadline. Between now and the beginning of 1999, however, the Agency has scheduled many opportunities for input from the full range of stakeholders likely to be affected by the new regulation.

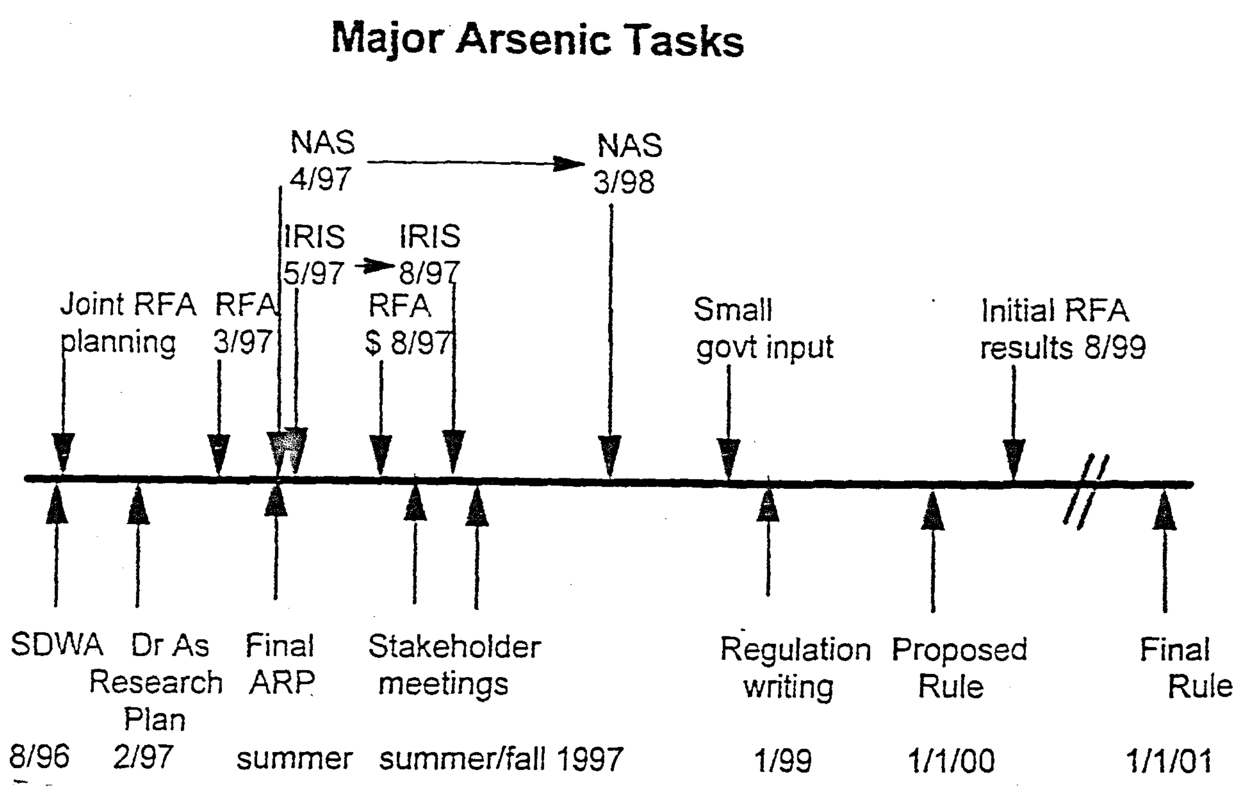


Figure 2. Schedule for completing arsenic-related activities in response to the Safe Drinking Water Act Amendments of 1996

To give the panel a clearer sense of how their contributions to an improved characterization of risk will fit into the broader rulemaking process, Mr. Taft presented the schematic diagram reproduced as Figure 3. In addition to the risk characterization being developed with the help of this panel and other experts in arsenic toxicology, writers of the proposed regulation will be guided by a number of considerations, including:

- The sufficiency of analytic methods currently available for arsenic, including their cost.
- The sufficiency of existing treatment technologies, including their availability at the small systems level.

- The relationship between the proposed standard and the range of arsenic concentrations occurring in different drinking water sources.
- The costs and benefits associated with the proposed regulation.
- Methods for implementing the new arsenic standard.

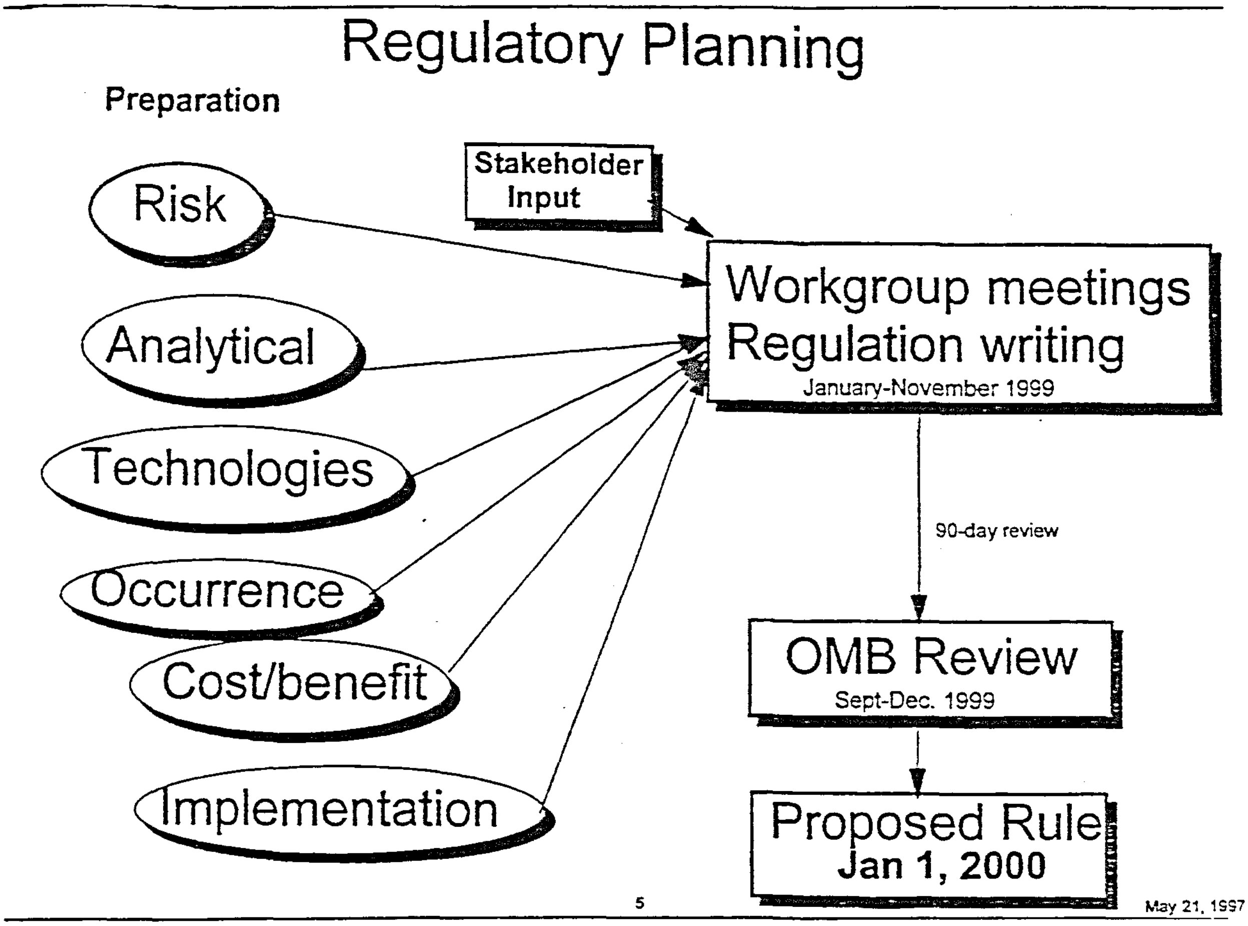


Figure 3. Risk characterization as one of several key factors that will inform the rulemaking process for arsenic

As in the risk characterization process, stakeholder input will be solicited in each of these other areas throughout the rulemaking process. Once a new regulation is drafted, it will be sent to the Office of Management and Budget (OMB) for administrative review before being formally proposed on January 1, 2000. Having established a framework within which many of these activities can proceed in parallel, the Agency believes that the statutory deadline is a challenging but realistic one.

Introduction, Background, and Charge to Panel

Dr. Jeanette Wiltse, EPA Office of Water

Dr. Wiltse began her presentation by comparing the risk assessment process to that involved in assembling a jigsaw puzzle. Because risk assessors never have all the information they would like to have, their task always involves trying to assemble the puzzle despite knowing that some of the pieces are missing. It is important, therefore, to squeeze as much information as possible out of the pieces that are available, including any inferences that can be drawn about the shape of missing pieces. Thus, by taking in this panel's description of one piece of the arsenic puzzle and the NAS panel's description of another, EPA hopes to gradually get a sense of how these and other pieces of the puzzle fit together and what the finished picture might look like.

In the case of arsenic in drinking water, EPA's review of the data began with a relatively clear understanding that arsenic acts a human carcinogen in the setting of inhalation exposure. Epidemiologic studies have also shown that exposure to arsenic via drinking water is a hazard, but the exposure levels in these studies are much higher than those likely to be encountered in U.S. drinking water supplies. The problem that EPA faces, therefore, is how to use the available data to assess and ultimately to manage the risks associated with exposures significantly lower than those at which health effects have been observed. Although Agency scientists are fairly confident that the upper limit of exposure should be lower than the 50 μg/L established on the basis of the old Public Health Service standard, they are less confident about precisely where this limit should be.

Another driving force behind the arsenic effort is the Agency's desire to update the information contained in the Integrated Risk Information System (IRIS) database, which is widely used by EPA, by regional EPA offices, and by state and local entities charged with implementing site-specific risk assessment and risk management efforts. In particular, the Agency would like to update the IRIS database to reflect recent advances in the understanding of mechanisms of carcinogenicity.

In convening this panel of experts to share their knowledge of the mode of action of arsenic as a human carcinogen, EPA hoped to forward both of these goals. In particular, the Agency is interested in the experts' views regarding three critical issues:

- What inferences can be drawn from the existing data related to the mode of arsenic carcinogenesis, and with what level of confidence?
- Are there specific modes of action that can be ruled out for arsenic, and with what level of confidence?

- What are the missing pieces in the arsenic puzzle, and what research is needed to fill these critical information gaps?

Answers to these key questions, in turn, will help the Agency in its efforts to determine which method of extrapolation is most appropriate for estimating the risks associated with exposure to arsenic at the low end of the dosage range. As stated in the 1996 *Proposed Guidelines for Carcinogen Risk Assessment,* the Agency's overriding preference is to use a biologically-based or case-specific model for extrapolations outside the observed range of dose-response data (U.S. EPA, 1996; Wiltse and Dellarco, 1996). When no biologically-based or case-specific model is available, however, the default procedure is to use a curve-fitting model. The criteria outlined in the *Proposed Guidelines* for choosing an appropriate curve-fitting model are summarized in Figure 4. In general, EPA proposes the use of a linear default when information about a carcinogen's mode of action either supports linearity or fails to support nonlinearity in the dose-response relationship. If there is evidence to support a nonlinear dose-response relationship, however, a more complex analysis is proposed; the goal of this effort, known as a margin of exposure analysis, is to provide the risk manager with as much information as possible about the risk reduction likely to be associated with each incremental reduction in the exposure limit. When mode of action information indicates that the dose-response is likely to involve both linear and nonlinear components, the proposed default is to present both the linear and margin of exposure analyses. An important goal of the workshop, therefore, will be to obtain guidance as to which of the models described in the *Proposed Guidelines* seems most appropriate given the current understanding of arsenic's carcinogenic mode of action.

17970 Federal Register / Vol. 61, No. 79 / Tuesday, April 23, 1996 / Notices

Data to Support:					
Biologically Based or Case-Specific Model	yes	no	no	no	no
Linearity		yes	no	yes	no
Nonlinearity		no	yes	yes	no
Extrapolation Used:	model	default--linear	default--nonlinear	default--linear and nonlinear	default--linear

Figure 4. Decisions on dose-response assessment approaches for the range of extrapolation (U.S. EPA, 1996)

Summary of Pre-Meeting Comments

Dr. Julian Preston, Chair, Expert Panel on Arsenic Carcinogenicity

Prior to the workshop, each member of the expert panel was given published and in- press articles on the biological and biochemical effects of arsenic (Appendix B). To give the group a common starting point for the ensuing discussion, Dr. Preston offered a brief summary of the pre-meeting comments submitted by individual panel members. The panelists' pre-meeting comments are reproduced in their entirety as Appendix C.

Dr. Preston began his overview by commenting on the distinction between mechanism and mode of action, which was reflected throughout the pre-meeting comments. Although it would clearly be desirable to have information about the specific mechanism through which exposure to arsenic leads to the development of a tumor, the fact is that this sort of mechanistic understanding remains elusive for most chemicals and most tumor types. It is often possible, however, to form meaningful conclusions about a chemical's mode of action, which is a much more general description of its effect on one or more biological processes. These conclusions, in turn, can often be used to predict the general shape of the dose-response curve.

Also, Dr. Preston reminded the panel that one of the stated aims of the workshop is to identify ways of reducing uncertainty in the arsenic risk assessment. Toward this end, he suggested that it might be particularly useful to EPA for the panel as a group to discuss suggestions raised in the pre-meeting comments of ways the Agency might go about addressing existing areas of uncertainty.

Focusing first on comments related to hazard identification, Dr. Preston noted that there seemed to be a consensus among panelists that arsenic does act as a human carcinogen, particularly in the setting of skin tumors. Although tumors of the urinary bladder, liver, kidney, and colon have also been reported, the role of arsenic as a carcinogen is more controversial for these other sites. Resolving the issue of single- or multi-site carcinogenicity could be useful, Dr. Preston suggested, since this distinction might suggest something about arsenic's mechanism of carcinogenicity (e.g., whether or not the mechanism involves some sort of mutagenic action).

One factor that panelists felt complicates the issue is that there is at present no good animal model for arsenic carcinogenicity. Although rodent studies have suggested that arsenic or one of its metabolites may act as a co-carcinogen, arsenic alone has not been demonstrated to cause tumors in any animal system. Because of this, animal data are of little value in efforts to extrapolate from high- to low-dose arsenic exposures, and there is considerable need for a marker that could be used to predict the likelihood of cancer based on something that can be observed at lower doses. Although arsenic-induced tumors themselves can and should be studied, it would be extremely useful to identify some sort of molecular signature or other surrogate marker that could be used to study earlier stages in the carcinogenic process.

Turning to comments more specifically related to the shape of the dose-response curve, Dr. Preston first described those factors that panelists identified as confounding the dosage side of the equation. It remains unclear, for example, which of the various species of arsenic (i.e., arsenite, arsenate, or the methylated metabolites) should be considered the "bad actor" in the tumor production process. If it is one of the metabolites, moreover, interspecies differences in arsenic metabolism would further complicate the extrapolation from animal to human health effects. For these reasons and others, Dr. Preston suggested that the issue of what constitutes dose would be an important part of the panel's discussion.

The panel also would need to consider the fairly broad range of responses that have been reported following exposure to arsenic. In addition to determining which of these are and are not relevant to the issue of carcinogenicity, it might be possible to draw inferences about arsenic's mode of action from the types of responses that have and have not been observed. For example, the fact that arsenic does not seem to produce point mutations in standard bioassays might suggest that a particular mode of action is or is not at play. Other responses to arsenic that might similarly point to a particular mode of action include the reported occurrence of chromosomal aberrations and sister chromatid exchanges, alterations in the methylation of genes and gene regions, and effects on DNA repair.

These various modes of action, in turn, might be useful in establishing the general shape of the dose-response curve. Point mutations, for example, are usually associated with a linear dose-response, while chromosomal aberrations, which require at least two lesions, are usually nonlinear. Changes in DNA methylation could produce a linear or nonlinear response, depending on the level at which it occurred; similarly, effects on DNA repair could produce either type of dose-response curve, depending on whether these effects occurred via a direct or indirect mechanism.

Based on its consideration of the various dose and response issues, the panel would by the end of the meeting attempt to come to some conclusion regarding the most feasible mode of carcinogenicity for arsenic, identifying not only those modes of action that seem most likely to be operating, but also those that can likely be ruled out. Among the candidate modes of action identified in the pre-meeting comments, Dr. Preston listed oxygen radical/stress response, effects on DNA repair, and effects on methylation. After considering the strength of the experimental evidence for these and other possible modes of action, the group's final task would be to determine whether the conclusions they had reached could or could not be used to support specific inferences about the shape of the dose-response curve.

Fundamentals of Carcinogenesis

Dr. Samuel Cohen, Member of Expert Panel

Dr. Cohen began by noting that the purpose of his presentation was to provide a brief overview of the general mechanisms of carcinogenesis as they are currently understood. In using a bioassay to study the carcinogenic effects of any chemical, two basic assumptions usually come into play: that the effects observed at high doses will also occur at low doses, and that chemicals that cause cancer in rodents will also cause cancer in humans. In the case of arsenic, however, there is no good animal model. As a result, the questions that have to be addressed all involve the relationship between the high doses at which human health effects have been observed and the lower doses that are typically encountered in drinking water.

To begin formulating hypotheses about a chemical's mode of action, it is useful to consider what we know about cancer itself. It is clear, for example, that genetic alterations are required for cancer to occur, and it is also well established that more than one genetic alteration is required. It makes no difference whether the tumor is caused by direct genetic inheritance or by some indirect insult, nor does it matter whether the tumor occurs in a human or in a laboratory animal; all tumors have in common the presence of two or more transmissible genetic alterations.

In addition, it has been known almost since the time DNA was first discovered that replication of the genetic material does not occur with 100 percent fidelity. Although rare, occurring at a rate of approximately one per every 10 billion nucleotides per replication, mistakes do occur. Since the total size of the human genome is estimated at 1 billion nucleotides, it is possible for an error-free replication to occur. In practice, however, errors occur with some regularity. Once described as "spontaneous errors," these anomalies are now known to result from a range of different mechanisms, including replication errors (mismatch repair), oxidative damage, depurination/depyrimidination, deamination, inappropriate alkylation, nitric oxide, exocyclic adducts, and others. These errors are almost always completely repaired; it is only when they are not repaired that a permanent mistake in the DNA occurs.

To increase the likelihood of developing cancer, a chemical can act in one of two ways: it can either damage DNA directly (as occurs in adduct formation or following exposure to radiation), or it can cause an increase in the number of cell divisions in the target cell population. In the case of arsenic, the latter effect appears to be most important; although there is little reason to believe that arsenic is active in directly damaging DNA, there is considerable evidence to suggest that arsenic causes an increase in the number of cell divisions occurring in certain tissues. It is this increase in cellular proliferation that indirectly leads to replication errors and ultimately to the development of cancer.

For the rate of cellular proliferation to increase, there must be an increase in the number of cell births, a decrease in the number of cell deaths, or both. A chemical can increase the number of cell births either by acting as a direct mitogen, as hormones and growth factors do, or by triggering a toxic response that is followed by regeneration, as most other types of chemicals do. To decrease the number of cell deaths, a chemical can either inhibit apoptosis, or it can inhibit the process of cellular differentiation.

The precise mechanism by which arsenic increases cellular proliferation is not known; certainly there is evidence that arsenic is toxic to some types of cells, and the hyperkeratosis that often precedes the development of skin tumors appears to involve a partial blocking of normal differentiation mechanisms. The effect of all of these changes, however, is to increase the overall number of DNA replications in arsenic-exposed cells, which also increases the likelihood of replication errors.

Turning to the various models that have been developed to explain the multistage process of carcinogenesis, Dr. Cohen first described the initiation/promotion model summarized in Figure 5. In this model, which was developed to explain the results of a series of experiments in mice, one substance acts as an initiator of the carcinogenic process, and another acts as a promoter. For a tumor to occur, cells must be exposed first to the initiator and then to the promoter. Exposure to either substance alone fails to generate tumors, as does exposure to the promoter before the initiator. Tumors form even if there is a substantial time gap between exposure to the initiator and exposure to the promoter, as long as exposure to the initiator occurs first and exposure to the promoter is not fractionated beyond some threshold value.

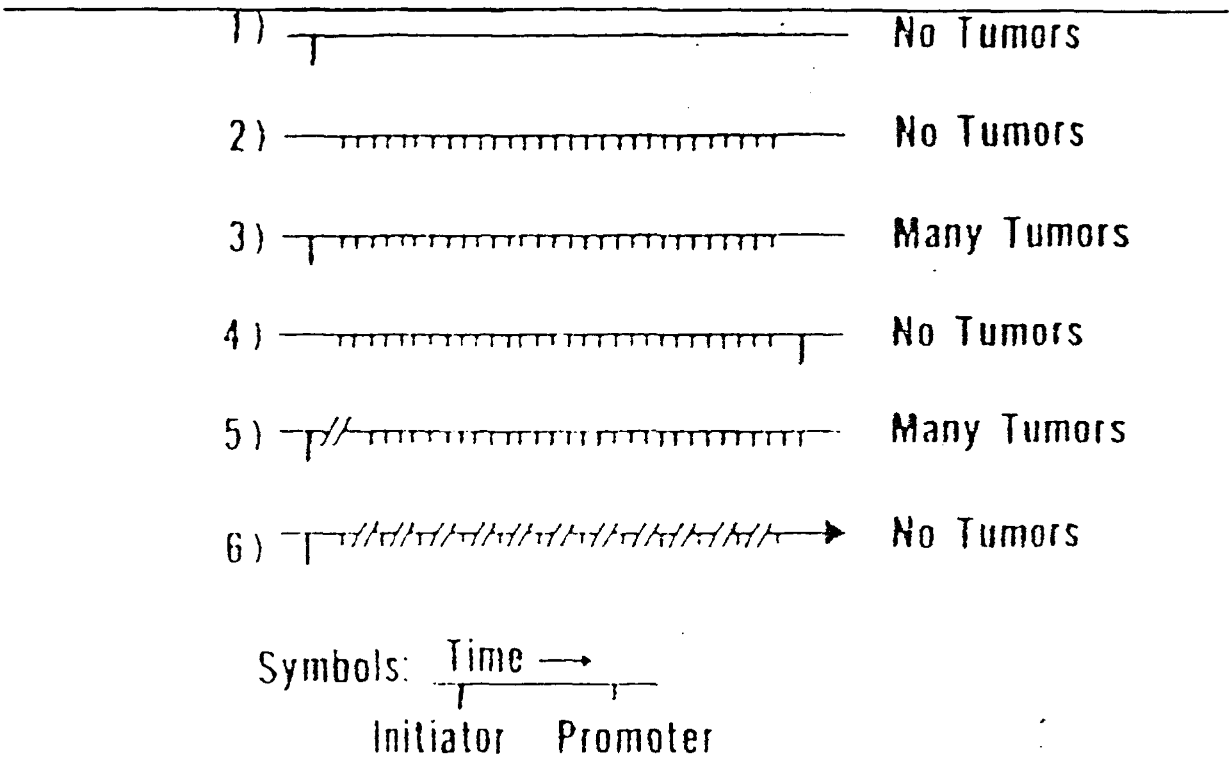


Figure 5. Initiation/promotion multistage model of carcinogenesis

Although the initiation/promotion model has been very useful in distinguishing between chemicals that interact directly with DNA, producing a defect in genetic memory, and those that exert their effects less directly, through nonmemory processes, its account of the process of carcinogenesis is overly simplistic. With the advent of longer-term bioassays, for example, it has become clear that administration of a promoter alone can cause a tumor to develop. Also, tumor incidence has now been shown to increase even when a promoter is administered before an initiator. Another problem with the initiation/promotion model is the notion of an initiator being a subcarcinogenic dose of a carcinogen, which raises the possibility of a threshold for genotoxicity.

A second multistage model for cancer, the Armitage-Doll model, is summarized by the equation in Figure 6. This model was developed to explain the tendency for tumor incidence to increase exponentially with age, which had been noted in human epidemiologic studies of lung, prostate, colon, and skin cancer. Where such an exponential increase occurs, it is possible to use the equation derived by Armitage and Doll to estimate the number of genetic events needed for the process of carcinogenesis to occur. Depending on the type of cancer, this number generally falls between four and seven.

ARMITAGE-DOLL MULTISTAGE MODEL

$$I(t) = N \lambda_0 \lambda_1 \ldots \lambda_{n-1} t^{n-1} / (n-1)!$$

$I(t)$ = incidence at time t

N = number of normal stem cells

λ = rate of transition between stages

n = number of stages

Figure 6. Armitage-Doll multistage model of carcinogenesis

The main problem with the Armitage-Doll model is that it fails to account for a number of tumor types, including a variety of childhood tumors (which occur in childhood or not at all), Hodgkins disease (which has one peak incidence in young adulthood and another late in life), and breast cancer (which peaks in the perimenopausal years and then again later in life). The reason is that the model relies on several assumptions that do not always hold true; namely, that the number of cells and the mitotic rate within a given tissue both remain constant throughout life.

A third multistage model for carcinogenesis was put forth in the early 1970s to explain the epidemiology of retinoblastomas. As Figure 7 illustrates, development of a retinoblastoma requires two genetic events, the first involving a shift from wild type to heterozygous and the second from heterozygous to homozygous defective alleles. These changes occur as a result of "spontaneous" errors in the genetic make-up of the retinoblastoma gene, rather than as a result of exposure to some exogenous carcinogen. Since the average rate for errors in DNA replication is estimated for the retinoblastoma gene at one in a million, the likelihood of both errors occurring in the same cell is roughly one in one trillion. A person who is born with one defective allele has a far greater chance of developing a retinoblastoma, since the likelihood of one additional error is obviously far higher than the likelihood of both errors occurring in a single cell. Altogether, though, the incidence of retinoblastomas is only about one per one million population.

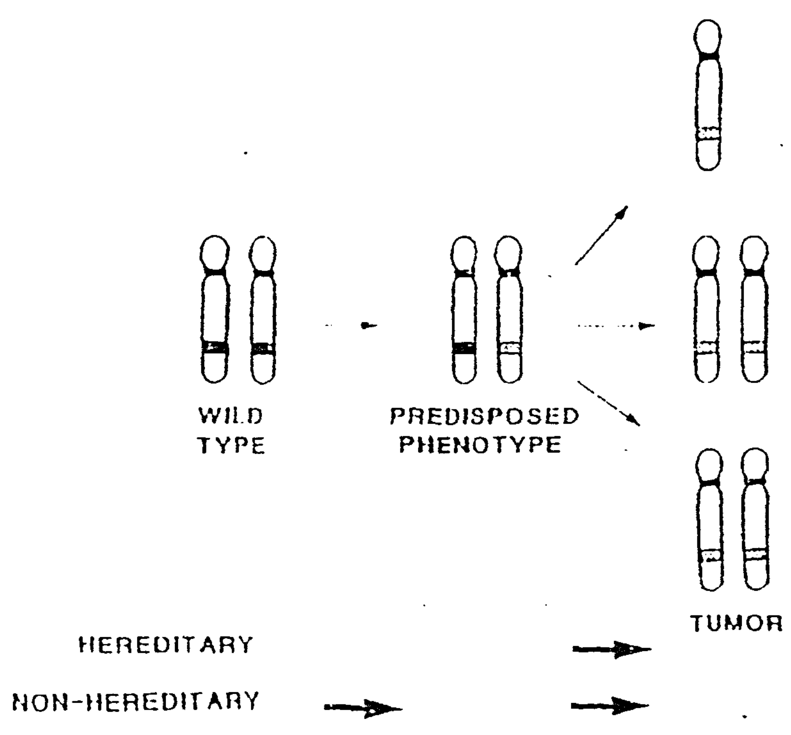


Figure 7. Multistage model of carcinogenesis based on the epidemiology of retinoblastomas

What is evident from the retinoblastoma model is that it is possible for two genetic events to occur simply through random errors in the replication process, without any direct damage to the DNA itself by an exogenous agent. Thus, an increase in the risk of cancer can occur in either of two ways: by direct damage to the DNA or by an increase in the rate of replication, which indirectly leads to an increase in the number of errors. Chemicals that increase the risk of cancer by interacting directly with the DNA are referred to as genotoxic, while those that increase risk by increasing the rate of replication are nongenotoxic.

Although generally true, it is not always the case that genotoxic carcinogens produce a linear dose-response curve and nongenotoxic carcinogens produce a nonlinear one. As an example of a situation in which this relationship does not hold, Dr. Cohen concluded his presentation by showing the graph reproduced as Figure 8, which illustrates the dose-response curves obtained for tumors of the liver and urinary bladder in mice exposed to 2-acetylaminofluorene (AAF). As one would expect from a genotoxic chemical, the dose-response curve for liver tumors (broken lines) was essentially linear at all ages; the dose-response for tumors of the urinary bladder (solid lines), however, is clearly nonlinear. The reason for this disparity is that, in addition to its genotoxic effects, AAF at these doses also affects cell proliferation in the bladder but not in the liver.

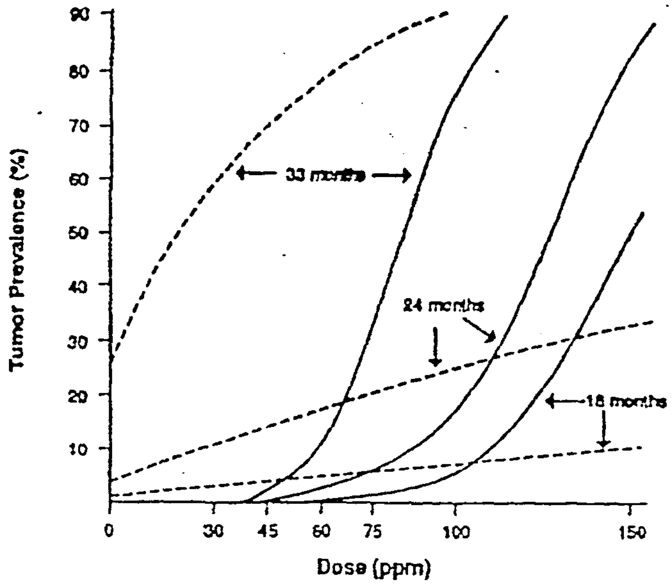


Figure 8. Dose-response curves for tumors of the liver (broken lines) and urinary bladder (solid lines) following exposure to AAF

IV. EXPERT PANEL REPORT

Executive Summary

Based upon pre-meeting review of the pertinent literature on arsenic carcinogenicity and other biological responses and pre-meeting comments on these issues, the expert panel developed a set of possible modes of action for arsenic carcinogenicity and their relative confidence levels. Modes of action that could be ruled out on the basis of experimental data were also discussed. The summary conclusions described here are placed in the context of the three issues identified in the meeting agenda (see Appendix D).

Issue No. 1: What do existing data tell us about arsenic's carcinogenic mode of action?

It was assumed that development of tumors requires genetic alterations and so information on mode of formation of these was considered in deliberations or cancer formation. Arsenicals do not induce point mutations, but they do induce chromosomal alterations (both structural and numerical), suggesting that the latter are formed by indirect effects upon DNA. Chromosome alterations can be produced by errors of DNA repair, DNA replication, and cell division. Their production and/or clonal expansion requires cell proliferation. All these housekeeping processes (repair, replication, division, and proliferation) could be influenced via transcription control (expression) mediated by DNA methylation changes, the extent of which could be determined in part by arsenic metabolism. Control could also be altered by direct interaction of arsenic with proteins involved in the housekeeping processes via vicinal dithiol binding. Changes in housekeeping processes (amount of fidelity) can lead to genomic instability, exemplified by chromosomal alterations. The formation of specific alterations in tumor suppressor genes, for example, could lead to tumor formation. Additional modes of action were deemed to include the production of oxidative radicals and co-mutagenic effects of arsenic with known chemical mutagens.

Issue No. 2: What is the level of confidence in conclusions regarding arsenic's mode of action?

There was a high level of confidence that chromosome alterations were induced by arsenicals and that specific chromosome alterations are involved in tumor formation. The process by which chromosome alterations (or genomic instability) are induced have a lower level of certainty. Effects of arsenic on DNA repair have been reported, including interactions with repair proteins. However, direct association of these on chromosome aberration induction is not known. Cell proliferation increases induced by arsenic have been reported to a limited extent, but the mechanism of this is unknown. Effects on DNA replication are also uncertain at this time. The role of oxidative damage in producing genetic alterations is known to be feasible, but is not well substantiated for arsenic exposures. While hypermethylation has been shown to be associated with tumor phenotype, little is known about arsenic-induced changes in methylation.

Issue No. 3: What are the dose-response implications of the mode of action understanding?

The induction of a chromosome aberration (deletion and translocation) requires two DNA lesions, in general. Thus, the dose-response curve is predicted to be nonlinear with dose, as is supported by experimental data. Cellular processes leading to aneuploidy, including effects on mitotic spindles and chromosome segregation, are predicted to be nonlinear with dose, as supported by some published literature. Increases in cell proliferation and hypermethylations are predicted to be nonlinear with dose, and experimental support is available in the case of cell proliferation. It is difficult to predict the role of co-carcinogenic effects of arsenic on dose-response curve shape without knowing the nature of the co-carcinogen. The evidence for this as a mode of action for tumor formation is limited. Taken as a whole, the proposed modes of action are predicted to produce nonlinear tumor responses as a function of effective dose. The exact nature of this nonlinearity will be enhanced by knowledge of metabolism and of carcinogenic/ mutagenic metabolites leading to a firmer assessment of effective dose.

Hazard Identification

The expert panel believes that it is clear from various epidemiologic studies that arsenic is a human carcinogen via the oral and inhalation routes. Target organs from oral exposure include primarily the skin and internal organs such as the liver, bladder, and kidney, while the target organ from inhalation exposure is primarily the lung.

Possible Modes of Action for Arsenic Carcinogenicity

The genetic toxicology of arsenic compounds has been reviewed (Lèonard and Lauwerys, 1980; Jacobson-Kram and Montalbano,1985; Wang and Rossman, 1996; Rossman, 1997). Based on available evidence, the expert panel concludes that arsenic and related compounds are not direct genotoxicants.

There is no evidence that arsenic compounds form DNA adducts, and incubation of plasmid DNA with arsenite, alone or in combination with UV light or H_2O_2 does not induce DNA strand breaks or alkali-labile sites (Rossman, unpublished data). The inability of arsenite to induce the bacterial distress ("SOS") system that is sensitive to DNA damage is also consistent with its lack of direct genotoxicity (Rossman et al., 1984). Since inorganic trivalent arsenicals are especially good inhibitors of enzymes containing vicinal sulfhydryl groups (Aposhian, 1989), arsenite is much more likely to affect chromosomes by binding to such groups on chromosomal proteins. Vicinal dithiols are common in the zinc fingers found in DNA binding proteins and transcription factors, and in some DNA repair proteins (Berg, 1990). Neither arsenite nor arsenate decreases the fidelity of DNA polymerization (Tkeshelashvili et al., 1980).

Unlike many carcinogens, arsenic compounds do not induce mutations in either bacterial or mammalian cells, at least under conditions of high survival (Rossman et al., 1980). Since this does not rule out the possibility of mutations caused by large deletions (which are either unselectable or lethal events in these systems), the mutagenicity of arsenite has also been also assayed in a transgenic line, G12. This Chinese hamster V79 cell line contains a single integrated copy of the *E. coli* xanthine-guanine phosphoribosyl transferase (gpt) gene and so can detect deletions as well as point mutations (Klein and Rossman, 1990). In G12 cells, arsenite induces point mutations only at relatively toxic concentrations; even at these high concentrations, moreover, the mutation rate is only twice background (Li and Rossman, 1991). When Meng and Hsie (1996) analyzed the mutants produced in another transgenic cell line treated with high concentrations of arsenite, the proportion of deletions was higher than in the spontaneous class; in this line, too, however, the mutation rate was only twice background.

Although low concentrations of arsenic and its metabolites alone do not cause mutations at single gene loci, these compounds do induce genotoxic effects. Theorized modes of action evaluated by the expert panel include:

- Chromosomal abnormalities
- Effects on DNA methylation

- Oxidative stress
- Effects on cell proliferation
- Co-carcinogenicity

The panel's conclusions regarding the weight of evidence for each of these possible modes of action, and implications for the shape of the dose-response curve, are summarized below:

Chromosomal Abnormalities. Although arsenic and arsenicals appear not to induce point mutations, there is considerable evidence that these compounds do induce abnormalities such as *changes in chromosome structure, changes in chromosome number, and sister chromatid exchanges* (reviewed in IARC, 1987; Rossman, 1994; and Rossman, in press). Chromosomal aberrations, including the induction of micronuclei, have been described in both *in vivo* and *in vitro* studies of rodents and humans (IARC, 1987; Jha et al., 1992; Dulout et al., 1996; Warner et al., 1994; and Larramendy et al., 1981). Similarly, arsenic-induced aneuploidy has been demonstrated *in vivo* and *in vitro* in human lymphocytes and in exfoliated bladder cells but not in buccal cells harvested from exposed humans (Vega et al., 1995; Warner et al., 1994; and Dulout et al., 1996). Sister chromatid exchanges are induced *in vitro,* but evidence for their occurrence in exposed human populations is more equivocal (Larramendy et al., 1981; Rasmussen and Menzel, 1997; Lerda, 1994; Nordenson et al., 1978; and Vig et al., 1984).

The great majority of chromosomal aberrations reported in the arsenic literature are of the chromatid rather than the chromosome type, indicating that they are formed during (S-phase) or after (G_2-phase) DNA replication. Some of the observed micronuclei have been shown to be a consequence of the loss of whole chromosomes (aneuploidy), while others are due to chromosomal acentric fragments (Dulout et al., 1996).

In vitro experiments have demonstrated that arsenic compounds are clastogenic in many cell types. In normal human fibroblasts, the potency for clastogenicity is: arsenite > arsenate > DMA (Oya-Ohta et al., 1996). In fact, >7 millimolar DMA is required for clastogenicity, whereas only 0.8 micromolar arsenite was clastogenic. Since the LD_{50}s for arsenite in human cells range from about 0.2 to 2.0 micromolar (Rossman et al., 1997), the panel felt that the genotoxic effects of arsenicals in human cells are unlikely to be due to DMA (which would only cause such effects if present in concentrations in the millimolar range).

In using the data on chromosomal abnormalities to define a mode of arsenic carcinogenicity, the panel thought the Agency should consider that such aberrations could be produced either by errors in DNA repair (failure to repair or incorrect repair) or errors in DNA replication (Preston, 1991). The former leads to chromosome-type aberrations in G_1 and chromatid-type in G_2; the latter leads to chromatid-type aberrations in the S-phase. In the panel's view, the fact that only chromatid-type aberrations have been observed following exposure to arsenicals, especially in G_0/G_1 exposed human lymphocytes, suggests that errors of DNA replication are involved in their formation.

Sister chromatid exchanges are produced by errors in DNA replication. The panel felt it notable that such abnormalities have not been reported in cells from human populations exposed to arsenic, perhaps indicating that an aberrant DNA replication process leading specifically to sister chromatid exchanges is not operating in arsenic-induced carcinogenicity.

Aneuploidy can be caused by any of a number of processes, ranging from chromosomal alterations to direct effects on the mitotic spindle or chromosome-moving components. The mechanism by which arsenicals induce aneuploidy is not known; however, studies of other chemicals suggest that the dose-response relationship for induced aneuploidy is nonlinear and may be best described by a threshold response (Elhajouji et al., 1995).

Arsenite causes cell transformation (but not mutation) in Syrian hamster embryo cells (Lee et al., 1985a), and similar results have been found using 10T1/2 mouse embryo cells (Landolph, 1994) and BALB/3T3 mouse embryo cells (Saffiotti and Bertelero, 1989). Arsenite also transforms human osteosarcoma cells to anchorage-independence (Rossman and Hu, unpublished). In SV40-transformed human keratinocytes, arsenite induces gene amplification at the dihydrofolate reductase (dhfr) locus, but does not cause amplification of SV40 sequences (Rossman and Wolosin, 1992). This suggests that arsenite does not induce signaling typical of agents that directly damage DNA (which induce SV40 amplification in this system), but rather could affect cellular gene amplification via checkpoint pathways such as those involving p53 (Livingstone et al., 1992). In fact, the panel felt that many of the genotoxic effects of arsenite are consistent with the type of genomic instability that could be expected to result from interference with p53-related pathways (Little, 1994) or other pathways involving DNA repair or cell cycle control.

Based on the lack of DNA adducts and the absence of point mutations, the panel concluded that chromosomal alterations induced by arsenicals are not the result of a directly mutagenic process. Rather, the panel believes it likely that acute high-dose exposure in *in vitro* cellular systems or chronic exposure to arsenic *in vivo* leads to a decrease in the fidelity of DNA replication (or perhaps a decrease in the efficiency of DNA repair) and a decrease in the efficiency of the cell division process that eventually results in chromosomal abnormalities. One area of uncertainty in this model is that an additional DNA response would be necessary to explain the observed lack of point mutations.

Although the precise mechanism is not known, such a response might involve the production of a DNA lesion that leads to chromosomal aberrations such as a secondarily produced DNA double-strand break or failure to repair damage to DNA, endogenous or exogenous (Elia et al., 1994).

While acknowledging that details of the mechanism remain obscure, the panel believes that the chromosomal abnormalities observed in arsenic-exposed cells occur via an indirect mechanism, probably one involving the derangement of some cellular housekeeping process, and that the dose-response curve for such indirectly produced chromosomal alterations is likely to be nonlinear. This proposition is supported by published data showing that in most cases the dose-response curves for chromosomal aberrations in cells exposed to arsenic are nonlinear (reviewed in Rudel et al., 1996). Thus, the panel had a fairly high level of confidence that such a mode of action, in which tumors form as a consequence of one or more chromosomal abnormalities, would produce a nonlinear dose-response curve.

Effects on DNA Repair. At nontoxic concentrations, arsenite acts as a co-mutagen and/or inhibitor of DNA repair. Arsenite has been found to enhance the mutagenesis of ultraviolet irradiation in *E. coli* (Rossman, 1981) and of UV, methyl methanesulfonate (MMS), and methyl nitrosourea (MNU) in Chinese hamster cells (Lee et al., 1985b; Li and Rossman, 1989a, 1991; Yang et al., 1992). Arsenic compounds inhibit the repair of DNA damage induced by x-rays and ultraviolet radiation (Snyder et al., 1989), the post-replication repair of ultraviolet-induced damage (Lee-Chen et al., 1992), and completion of the repair of MNU-induced damage (Li and Rossman, 1989a). The theory that arsenic compounds inhibit DNA repair is also supported by findings that arsenic compounds potentiate x-ray and ultraviolet-induced chromosomal damage in peripheral human lymphocytes and fibroblasts (Jha et al., 1992), alter the mutational spectrum (but not the strand bias) of ultraviolet-irradiated Chinese hamster ovary cells (Yang et al., 1992), and synergistically enhance chromosomal aberrations induced by diepoxybutane, a DNA cross-linking agent (Wiencke and Yager, 1992).

Although there is one report that arsenite treatment causes an inhibition of pyrimidine dimer removal in human SF34 cells after ultraviolet irradiation (Okui and Fujimara, 1986), most studies have found interference with a later (post-incision) step in the DNA repair process. The inhibition by arsenite of the completion of DNA excision repair appears to occur via effects on DNA ligation (Li and Rossman 1989b; Lee-Chen et al, 1994); however, neither DNA ligases nor DNA polymerase alpha or beta can be inhibited by arsenite at concentrations many times higher than those shown to inhibit DNA repair in cells (Li, 1989; Li and Rossman, 1989b; E. Snow, personal communication). Thus, the observed effects on DNA repair do not seem to be mediated via an arsenite-induced inhibition of DNA repair enzymes (ligases or polymerases), although effects on accessory proteins (if any) have not been tested. Rather, arsenite may affect cellular control of DNA repair processes, possibly through its effects on p53 expression (see discussion of DNA methylation, below).

Effects on DNA Methylation. Abnormalities in cytosine DNA methylation have recently emerged as a common molecular change in a variety of human tumors (Counts and Goodman, 1995; Jones, 1996; Issa et al., 1997; Baylin et al., 1997). When it affects the promoter region of expressed genes, hypermethylation is associated with transcriptional silencing of the involved gene (Meehan et al., 1992; Eden and Cedar, 1994). Several tumor suppressor genes have been shown to be transcriptionally inactivated in human tumors without detectable coding region mutation, but in association with promoter methylation. Therefore, hypermethylation can drive carcinogenesis either by inactivating multiple genes (e.g., tumor suppressor genes, angiogenesis inhibitors, and so forth), or by inactivating genes involved in DNA repair, with resultant genetic instability. Promoter hypermethylation has been found in most tumor types examined and therefore is a plausible mode of action for carcinogenesis in general.

The involvement of DNA hypermethylation in the mode of action of arsenic carcinogenesis is supported by both theoretical considerations and preliminary experiments. Arsenic is primarily nonmutagenic, and hypermethylation, which provides an alternative to coding region mutations for the inactivation of tumor suppressor genes, appears to be an attractive mode of action for nonmutagenic chemicals (Costa, 1995). In fact, nickel, another nonmutagenic carcinogen, has been shown to induce hypermethylation of an integrated gene construct in mammalian cells (Lee et al., 1995).

In one study that has addressed the issue of arsenic-induced changes in methylation directly, lung cancer cells in culture were exposed to increasing doses of arsenic. In this study, arsenic exposure was investigated as a cause of increased overall methylation levels and increased methylation of the p53 tumor suppressor gene promoter, including tracts of non-CpG cytosine methylation that may represent a "signature" abnormality for arsenic exposure (Mass and Wang, 1997); arsenic exposure has also been found to cause an increase in cytosine-DNA methyltransferase activity (Mass, personal communication). These data support a role for abnormalities in DNA methylation as a mode of action of arsenic carcinogenesis.

These observations must, however, be viewed and interpreted with some caution. There is no evidence that hypermethylation of gene promoters is an exclusive or unique mode of action for arsenic carcinogenesis. In fact, such methylation abnormalities have been described in both spontaneous tumors and tumors induced by mutagenic carcinogens (Issa et al., 1996). Furthermore, no studies of DNA methylation abnormalities have been conducted in arsenic-induced tumors, and hypermethylation of the p53 gene promoter has not been reported in any human tumor. Thus, the panel's level of certainty for hypermethylation as a mode of action for arsenic carcinogenesis is based primarily on general principles of carcinogenesis and limited preliminary experiments, and may be considered as "good" (though not definitive).

To assess the implications of this mode of action for the shape of the arsenic dose-response curve, it is instructive to consider the potential mechanisms of arsenic-induced hypermethylation. In the panel's view, two general mechanisms seem plausible. In the first, arsenic-induced methylation could change the relative levels of S-adenosyl methionine (SAM) and S-adenosyl homocysteine (SAH). These changes, in turn, could modulate the relative activity of cytosine methyltransferase, resulting in aberrant methylation. However, cellular SAM/SAH pools are relatively large and serve all transmethylation reactions in the cell (Chiang et al, 1996). Thus, it appears unlikely that arsenic-induced methylation would sufficiently affect these pools to influence genomic methylation. Furthermore, profound changes in SAM/SAH concentrations would probably affect essential cellular processes (e.g., polyamine metabolism) before influencing DNA methylation. Nevertheless, it is quite clear that this mechanism would generate a nonlinear or threshold type of dose-response curve for arsenic carcinogenesis.

Alternatively, arsenic-induced methylation could affect chromatin structure and/or DNA tertiary structure by interacting with DNA binding proteins (Lewis et al., 1992) or, perhaps, by binding directly to DNA. This appears to be a plausible mechanism for arsenic carcinogenesis, but there are no data to support it at the present time. If demonstrated, however, such a mechanism (involving arsenic-protein interactions) would also be associated with a nonlinear dose-response curve.

Thus, while the panel's level of confidence for a specific role for arsenic-induced changes in DNA methylation in tumor induction is low, due to the absence of relevant experiments, the panel believes that all proposed mechanisms for this mode of action would likely be associated with a nonlinear dose-response curve.

Oxidative Stress. The generation of active oxygen species or their accumulation due to decreased scavenging has been implicated in several classical experiments of tumor promotion (Slaga et al., 1981; Klein-Szanto and Slaga, 1982). In addition, there is a well-known body of literature that points to free radical generating substances as having promoting effects due to their ability to induce cell proliferation (eventually through the induction of ornithine decarboxylase). Free radicals may also directly damage DNA (Cerrutti, 1985; Pryor, 1986).

There is a body of evidence implicating free radicals as significant factors in the development of arsenic-induced neoplasia. Yamanaka et al. (1989) found that dimethylarsine, a volatile metabolite of DMA, is mutagenic in *E. coli*. Subsequently, Yamanaka and Okada (1994) reported that DMA induced lung-specific DNA strand breaks in mice and rats via the peroxy radical and other active oxygen species produced during the metabolism of DMA. It was suggested that this might explain how arsenite induces lipid peroxidation in various rat tissues (Ramos et al., 1995). Given that Yamanaka et al. (1989) and Yamanaka and Okada (1994) used high concentrations of DMA, however, it is unlikely that DMA would be present in high enough concentration in mammalian cells exposed to inorganic arsenic compounds to cause mutagenesis (Vahter and Marafante, 1989).

The concept that arsenite induces oxidative stress is supported by a number of findings. The addition of superoxide dismutase to the culture medium has been shown to block arsenite-induced genotoxicity in human lymphocytes (Nordenson and Beckman, 1991), and Vitamin E (alpha-tocopherol) protects human fibroblasts from arsenite toxicity (Lee and Ho, 1994). An x-ray sensitive, catalase-deficient CHO cell variant is hypersensitive to killing and micronucleus induction by arsenite, and micronucleus induction can be blocked by catalase (Wang and Huang, 1994). In addition, arsenite induces a number of proteins that are induced by and protect against oxidative stress, including metallothionein (Albores et al., 1992) and heme oxygenase (Keyse and Tyrrell, 1989), and this induction, too, is blocked by antioxidants. Expression of metallothionein affords some protection against arsenite toxicity, even though metallothionein does not have a high affinity for arsenite (Goncharova and Rossman, 1995), and glutathione depletion increases the toxic and clastogenic effects of arsenite in cultured human fibroblasts (Oya-Ohta et al., 1996).

Genotoxicity can also occur via oxidative actions other than DMA peroxyl radical formation. Oxidative effects of arsenite may be caused by glutathione depletion. Arsenite readily reacts with glutathione, and glutathione is required both for reduction of arsenate to arsenite and in the reductive methylation of arsenite to DMA (Scott et al., 1993). In the case of fibroblasts and other cells that do not appear to methylate arsenic and thus cannot generate the DMA peroxy radical, the most likely mechanism of oxidant stress would be via depletion of glutathione after arsenite treatment. However, this also may be a high-dose effect, since cells normally contain millimolar concentrations of glutathione. Trivalent inorganic arsenite as well as organic arsenicals can inhibit glutathione reductase (Styblo et al., 1997), which would also lead to oxidant stress in the cell.

There is, however, another interpretation of antioxidant effects on arsenite genotoxicity. Endogenous oxidants play an important role in "spontaneous" mutagenesis (Goncharova et al., 1996). Thus, the addition of antioxidants to the medium reduces oxidant stress and oxidative DNA damage, which is thought to be responsible for most of the deletions seen in the spontaneous mutant spectrum (Joenje, 1989). In the presence of antioxidants, therefore, there might be fewer DNA lesions whose repair could be interrupted by arsenite and therefore fewer genotoxic events induced by arsenite.

Effects on Cell Proliferation. As noted previously, there is good agreement that arsenicals do not interact directly with DNA. An alternative mode of carcinogenic action is increased cell proliferation in the pluripotential cells of one or more target tissues, due either to an increase in the number of cell births and/or a decrease in the number of cell deaths. Although not examined extensively, there is both *in vivo* and *in vitro* evidence supporting this mode of action for arsenic-induced carcinogenesis.

In humans, exposure to high levels of arsenic and related compounds produces arsenical keratoses, usually on the palms and soles of the feet, which may evolve into invasive squamous cell carcinomas (Lever and Schaumburg-Lever, 1983). Characteristic features of these keratoses include dysplastic changes in the squamous epithelium that reflect a decrease in differentiation and an increase in the number of cell divisions. No articles were found, however, examining the role of cell proliferation in the preneoplastic stages of cancer of internal organs in humans that might be associated with arsenical exposure (lung, bladder, liver, kidney).

In rodents, oral administration of DMA after exposure to one or more genotoxic carcinogens results in increased incidences of tumors of the lung, bladder, liver, kidney, and thyroid (Yamanaka et al., 1996; Yamamoto et al., 1995; Wanibuchi et al., 1996). Administration of DMA without prior exposure to genotoxic carcinogens results in increased cell proliferation in the bladder (Wanibuchi et al., 1996), liver (Yamamoto et al., 1995), and kidney (Murai et al., 1993); proliferation rates in the lung and thyroid have not been specifically examined. Increases in cell proliferation have been detected in the form of dysplasia on histopathologic examination, in increased labeling indices following pulse bromodeoxyuridine, and by changes in ornithine decarboxylase activity. Cells have also been reported to exhibit increased proliferation *in vitro* following the addition of certain arsenicals to the culture medium (Rossman, personal communication). In the kidney, cytotoxicity has been demonstrated (Murai et al., 1993).

Although the mechanism through which exposure to arsenicals induces increased cell proliferation remains uncertain, the most likely pathway includes cytotoxicity followed by regenerative proliferation (Murai et al., 1993; Wanibuchi et al., 1996). The dose-response for this effect is nonlinear, with no increase at low doses and increasing proliferation as the dose increases. At very high doses, toxicity becomes a limiting factor, resulting in no further increases in proliferation. A nonlinear dose response is usually observed for increased cell proliferation related to chemical exposure.

The panel had some confidence in increased cell proliferation as a mode of action for arsenic carcinogenesis, but the amount of data available to support such a hypothesis is quite limited. Although the panel felt that additional quantitative studies could be done relatively easily in animals to determine the shape of organ-specific dose-response curves for arsenic-induced increases in cell proliferation, there was concern that extrapolation of these results to humans might be difficult because of species differences in the distribution, metabolism, and excretion of arsenic, as well as differences in tissue responsiveness.

Co-Carcinogenicity. The notion that arsenic and related compounds could be co-carcinogens but not complete carcinogens is supported by several observations. Although there are at least three published studies in which DMA has been reported to induce bladder, liver, or lung neoplasms when administered in combination with other carcinogens, there is no animal model in which arsenic alone has been shown to be carcinogenic. Similarly, despite their demonstrated ability to enhance the mutagenicity of other compounds (probably by altering the DNA repair pathways), arsenic compounds generally yield negative results in standard assays of mutagenesis such as the Ames test. Co-carcinogenesis is also suggested in some human epidemiologic studies, which have found a higher incidence of lung neoplasms in arsenic-exposed smokers than in arsenic-exposed nonsmokers (Hertz-Picciotto et al., 1992; Chiou et al., 1995) and in the subset of arsenic-exposed miners who were also exposed to radon gas (Xuan et al., 1993). Although not as clearly demonstrated, a similar cooperation is probable in the development of bladder cancer in populations exposed to arsenic and tobacco smoke.

Skin cancer in humans is probably not due to synergism between arsenic and ultraviolet radiation, since carcinomas in arsenic-exposed individuals frequently occur in areas of the skin that are not typically exposed to sunlight. Several attempts to develop an animal model of skin cancer using arsenic as either a complete carcinogen or as a tumor promoter have failed. Relatively few experimental protocols have been tested, however, leaving open the possibility that these failures can be attributed to inadequate species/strain selection, the schedule of arsenic administration, or other aspects of the experimental protocol.

Conversely, moderate success has been attained in experiments using arsenic to induce internal organ tumors in rodents (Yamamoto et al., 1995; Wanibuchi et al., 1996; Yamanaka et al., 1996). These studies have in common their use of DMA as a promoting agent after an "initiating" dose of another known carcinogen (a cocktail of nitrosamines or N-butyl-(4-hydroxybutyl)-nitrosamine (BBN) to induce bladder cancer and 4-nitroquinoline-1-oxide (NQO) to induce lung cancer). In these reports, DMA alone did not produce tumors, even at high doses (100-400 ppm); when combined with other carcinogenic compounds, however, DMA produced bladder tumors even at relatively low concentrations (10-25 ppm).

Further support for arsenic co-carcinogenesis comes from *in vitro* mutagenic experiments combining mammalian cell exposure to arsenite with exposure to ultraviolet irradiation, NMU, and methylmethane sulfonate (Lee et al., 1985b and 1986; Li and Rossman, 1989a, 1989b, and 1991; Yang et al., 1992). Arsenic also acts as a co-carcinogen in the clastogenesis of mammalian cells using ultraviolet radiation, x-rays, and diepoxybutane (Jha, 1991; Wiencke and Yager, 1992). It has been hypothesized that the observed co-mutagenesis is due to the effects of arsenic on DNA repair activity, such as DNA ligase II, through which arsenic interferes with the completion of DNA excision repair (Li and Rossman, 1989a and 1989b; Lee-Chen et al., 1994). However, as discussed above, DNA ligases are not particularly sensitive to arsenite.

It is difficult, given the present state of knowledge, to draw conclusions about the shape of the dose-response curves for cancer with arsenic acting as a co-carcinogen. The other agents involved will need to be identified and the nature of the interaction with arsenic established.

Implications for Arsenic Risk Assessment

Once a compound is identified as a potential human carcinogen, as arsenic has been, the next step in the risk assessment process is to identify, to the extent possible, the likely dose-response for humans over as much of the dose range as possible. This can be done in two ways: empirically or mechanistically.

Empirical data consist of dose-response information related to tumor production in animals and humans, as well as information on surrogates of both dose and effect. The use of surrogates may allow an extension of the dose-response data to the lower-level exposures that are of interest for regulatory purposes. In the case of arsenic, however, reliable surrogate dose-response information is not available. A recent reexamination of the Taiwanese study may indicate a nonlinearity in the observed data, but the panel's confidence in this conclusion is low due to both the difficulties in defining exposure in this population and the heavy confounding of exposure with age.

It is not clear exactly what the mechanism of arsenic carcinogenicity is, nor even which mode of action is operative. Several different modes of action have been postulated, however, and the panel concluded that each of them is both theoretically plausible and realistic from an operational point of view. There is, however, very little empirical data to support any one mode of action over the others. The panel believes that it is also plausible that more than one mode of action may be operating at different dose levels or even at the same dose.

The panel was able to conclude, however, that one important mode of action is unlikely to be operative for arsenic. The panel agreed that arsenic and its metabolites do not appear to directly interact with DNA. Had there been evidence for such a mode of action, it would likely have led to the conclusion that tumor induction was linear with dose over the dose range from the lowest point of observation for tumors. The conclusion that there does not appear to be any direct interaction of arsenic with DNA does not rule out a linear dose-response relationship at lower doses. However, all identified modes of action would lead to nonlinear responses for cancer.

There was a consensus among the panel that for each of the modes of action regarded as plausible, the dose-response would either show a threshold or would be nonlinear. Which of these shapes is the most likely at low doses was not discussed at any length, however, since under the new EPA Cancer Risk Assessment Guidelines, it makes little difference whether a carcinogen has a true threshold or simply exhibits nonlinear behavior at low doses. Additionally, the data needed to resolve this question are not available, except in generic form. It was clearly the consensus of the expert panel, however, that the dose-response for arsenic at low doses would likely be truly nonlinear—i.e., with a decreasing slope as the dose decreased. However, at very low doses such a curve might effectively be linear but with a very shallow slope, probably indistinguishable from a threshold.

Potency, or risk per unit dose, can be estimated from various study populations, but, as with any epidemiologic data, biases in the estimates obtained in this way are possible in both directions. In the Taiwanese study, for example, biases associated with the use of average doses and with the attribution of all increased risk to arsenic would both lead to an overestimation of risk. For dose estimates, this bias reflects the fact that despite a distribution of doses in the population, those individuals exhibiting effects would tend also to be those who received the highest doses; because of this, deriving an average dose based on affected individuals would to some extent bias risk estimates upward. Similarly, attribution of the total excess risk in the population to arsenic exposure alone could also be expected to inflate the estimate of risk if the population is also characterized by other risk factors such as smoking, excess exposure to sunlight, nutritional status, and so on. Other confounders, such as reduced animal fats in the diet, could produce a negative bias in the risk estimate.

Sources of Uncertainty

During the course of its deliberations, the expert panel identified several major sources of uncertainty that the Agency should take into account in assessing the risks associated with exposure to arsenic and related compounds. These include:

- Questions related to the speciation of arsenic and related compounds, including which species do and do not function as "bad actors" in the process of carcinogenesis.
- Questions related to the role of methylation in arsenic metabolism, including the saturability of methylation mechanisms.
- Questions related to the lack of a suitable animal model for arsenic carcinogenicity.

Each of these issues is discussed in some detail below.

Speciation. The issue of whether the methylated arsenic species MMA and DMA contribute to genotoxicity when exposure is to inorganic arsenic compounds has not been completely resolved, but it appears unlikely that the methylated species play a major role. Most studies using DMA have found that very high concentrations of this metabolite are needed to produce a genotoxic effect (i.e., approximately two orders of magnitude higher than the concentration of arsenite at which genotoxicity is observed). Relatively few studies of the genotoxicity of MMA have been conducted.

Cells that do not methylate arsenic compounds convert arsenate to arsenite and then excrete the arsenite via an efflux pump (Wang et al., 1996). Because arsenite is considered to be the most likely carcinogenic form of arsenic, there is more information on its genotoxicity than on that of other species. In general, arsenate is at least an order of magnitude less potent as a genotoxicant than is arsenite.

Finally, although arsenate, arsenite, methylarsonic acid containing As^{V} (MMA^{V}) or dimethylarsinic acid containing As^{V} (DMA^{V}) are the most recognized of the arsenic species, MMA^{III} and even DMA^{III} are certainly intermediates in arsenite methylation. Although these substances have been chemically synthesized and are available, they have neither been measured nor isolated in mammalian systems, and no studies of their mutagenic or carcinogenic properties have been performed.

Arsenic Metabolism. With the recent purification and characterization of arsenite methyltransferases (Zakharyan et al., 1995 and 1996), meaningful experiments to investigate the role of metabolism in inorganic arsenic carcinogenicity could be performed. These enzymes, which are found in the liver of rabbit, rat, mouse, hamster, pigeon, and rhesus monkey, are deficient in the marmoset monkey, tamarin monkey, squirrel monkey, chimpanzee, and guinea pig (Zakharyan et al., 1996; Healy et al., 1997; Aposhian, 1997). One of the major implications of these observations is that since there are species that do not methylate inorganic arsenic, arsenic methylation is not the major route of inorganic arsenic detoxification for mammals. Nonspecific binding to thiol-containing macromolecules has been suggested as an alternative mechanism for the detoxification of arsenic and related compounds.

Based on studies of the concentration of methylated arsenic species in particular tissues, it is often stated in the literature that methylation of arsenic and related compounds takes place only in the liver. Recently, it has been demonstrated that, in the male mouse, the specific activity of arsenite methyltransferases varies by organ, with activity in the testis > kidney > liver = lung (Healy et al., in press). However, such activity has not yet been demonstrated in human liver. Whether this is due to the unavailability of fresh human liver (i.e., tissue removed within one hour of death), to the absence of measurable amounts of an inducible enzyme in the livers of individuals not exposed to large amounts of inorganic arsenic, or to other factors is not known at the present time.

Another issue that remains controversial is the extent to which inorganic arsenic methylation varies as a function of exposure to arsenic in drinking water. On the one hand, studies of the urine of occupationally exposed humans, humans exposed to elevated doses of inorganic arsenic experimentally or via drinking water/food, or in humans exposed to much less inorganic arsenic in the general environment showed no major differences in the mix of arsenic metabolites compared to controls (Vahter, 1983; Hopenhayn-Rich et al., 1993; Mushak and Crocetti, 1995). These findings have been interpreted as suggesting the absence of a threshold for arsenic methylation, at least over the range of exposures studied.

Other reports, however, have suggested a threshold for arsenic methylation, which would imply that the dose-response curve for arsenic-induced cancer is sublinear at low doses (Carlson-Lynch et al., 1994; Beck et al., 1995). With increasing arsenic exposure, for example, an increasing percentage of MMA and a decreasing percentage of DMA are found in the urine (Hopenhayn-Rich et al., 1996; Del Razo et al., 1995). This phenomenon may be due to an inhibition in the methylation of MMA to DMA, as has been suggested by *in vitro* experiments using liver cells incubated with excess inorganic arsenic (Buchet and Lauwerys, 1985). However, these studies were performed in the rat and may not be applicable to humans, since there is known to be a large species diversity in the properties of arsenite methyltransferases (Aposhian, 1997), and properties of the human enzymes have not yet been fully elucidated.

It has been proposed that saturation of the methylation pathway for arsenic can be detected through a change in the ratio of urinary MMA to DMA (Beck et al., 1995). Consideration of ratios can be misleading, however, since a ratio often exaggerates a result. In this case, for example, the numerator (MMA) is becoming larger while the denominator (DMA) is becoming smaller. Perhaps the relationship between dose level and arsenic methylation would be more meaningful if assessments were based on the combined urinary excretion of MMA and DMA.

Assessments of the urinary excretion of MMA and DMA in population groups with varying levels of exposure to arsenic are problematic, however, since the exact daily arsenic dose, including the fractions absorbed from drinking water, food, and air, is seldom determined. Because of this, the results of studies involving human volunteers (Mappes, 1977; Tam et al., 1979; Buchet et al., 1981a, b) should be viewed with caution. Exposure conditions in these studies are not identical with normal exposure events and the number of subjects is typically small. In addition, many of the older studies failed to adequately address issues of validation and quality control, which casts additional doubt on their significance.

Further complicating the issue of arsenic methylation is the fact that decreases in the percent of DMA in urine at higher exposure levels have not always been found. For example, Andean women exposed to arsenic in the drinking water at a concentration of 200 μg/L had a higher percentage of urinary DMA than women exposed to less than 15 μg/L (Vahter et al., 1995). Similarly, in studies of native children drinking water containing arsenic in the Andean village and in Chaco province, the percentage of urinary DMA increased with increasing urinary concentration of methylated arsenic (Concha et al., 1997).

Animal Model. It is also important to keep in mind that in general experimental animals are less sensitive than humans to the toxic effects of arsenic. Because of the extensive species differences in both the amount of arsenite transferase in the liver and the amount of arsenic species excreted in the urine, the panel recommends that human tissues be used wherever possible for studies of arsenic carcinogenicity. The availability of freshly harvested human tissue is currently very limited; not only is the tissue expensive when available, but the logistics of obtaining it are extremely time consuming. To address questions dealing with the metabolism, mutagenicity, and carcinogenicity of arsenic species, however, access to human tissue is urgently needed.

Many investigators have studied the mutagenic and carcinogenic effects of arsenic in rats. The relevance of these results to humans is unclear, however. Among animal species, the rat is unique in its tendency to accumulate DMA in red blood cells; in addition, the rat excretes unusually large amounts of arsenic in bile. Because of the rat's unique handling of DMA, in fact, the National Research Council (1977) has recommended that this species not be used to study arsenic metabolism.

Based on its review of available data, the panel concluded that at the present time there does not appear to be any good animal model for studying arsenic carcinogenesis. Most investigators have used the excretion of arsenic species in urine (inorganic arsenic, MMA and DMA) as a measure of exposure; however, such profiles vary a great deal among species. For example, humans excrete significant amounts of MMA in urine, but most animals do not. Using MMA excretion as a criterion, the rabbit is considered the best animal model for arsenic biotransformation in humans. Using this same criterion, the hamster appears to be the second best animal model.

Research Needs and Priorities

Although not specifically requested to do so by EPA, the expert panel also discussed research efforts that might be undertaken to reduce the uncertainty associated with the current level of understanding of arsenic's mode of carcinogenic action.

Given the uncertainties surrounding animal models and cell culture experiments, the panel agreed that the most revealing studies would probably involve comprehensive genetic analysis of tumors from humans exposed to arsenic, compared with tumors from a control group. In addition to addressing the role of methylation in arsenic carcinogenicity, such studies might also involve comparative genomic hybridization (to determine the levels of chromosomal amplification/deletion), sequencing of specific genes (e.g., p53, Patched) and, perhaps, allelotype analysis.

The panel also agreed that there is a continuing need for large, carefully designed epidemiologic studies looking at both histopathological and molecular endpoints. In these studies, it appears important to carefully quantify arsenic exposure and to pay careful attention to confounding factors such as other exposures in drinking water and the smoking habits of the study population. The panel felt that such studies might have more power and be more useful in considering the impact of lower doses of arsenic if an endpoint other than cancer could also be measured. In particular, evaluation of preneoplastic lesions such as hyperkeratosis or proliferation of normal skin cells should be strongly considered.

If these histologic and epidemiologic studies were able to identify a common early abnormality or "signature" lesion in arsenic-exposed individuals, sensitive tests could be devised to detect these in skin biopsies and/or bioassays of other tissues (blood, urine). Such a lesion might also serve both as a useful molecular marker of exposure and as an endpoint in molecular epidemiologic studies. Therefore, if a large epidemiologic study is planned before the molecular nature of arsenic-related tumors is fully elucidated, the panel recommends that relevant specimens (skin, blood, urinary sediment) be collected and stored for future studies of this issue.

The panel also identified specific types of experiments that might be undertaken to increase the weight of evidence for one or more of the plausible modes of action for arsenic. To further explore the role of chromosomal abnormalities in arsenic carcinogenesis, for example, the panel felt that studies of long-term, low-dose exposures in an appropriate animal model (mouse, hamster, or rabbit), perhaps using fluorescence *in situ* hybridization techniques to assess transmissible chromosomal aberrations, might be useful. Experiments using mutant cells or transgenic rodents might also be helpful in this regard.

To further explore the role of DNA methylation as a target for arsenic carcinogenesis, the panel felt that studies examining promoter methylation at multiple gene loci (p53, P16, Patched, etc.) in skin tumors of patients exposed to high levels of arsenic in the diet and drinking water might be useful, particularly if these data were compared to control tumors of the same histologic type that were not related to arsenic exposure (e.g., sun-induced skin tumors). If a higher rate of promoter methylation were found in arsenic-related skin tumors, earlier-stage lesions might be particularly useful in revealing a mode of action for arsenic. In addition, genomic sequencing of the p53 promoter and other genes might confirm the presence of non-CpG methylation events, which in turn might represent a signature lesion that could be used as a biomarker for arsenic exposure. Finally, it might be of interest to measure DNA-methyltransferase levels and overall methylation levels in arsenic-induced tumors, although these might be considerably less specific.

Further studies to elucidate the role of arsenic as a co-carcinogen should use human populations with well-defined exposures to arsenic and other putative carcinogens (e.g., tobacco consumption). Using modern tools of molecular epidemiology, such studies might be used to identify precursor lesions and neoplasms of skin, bladder, and lung.

In addition, the panel thought that it would be very useful for animal studies of arsenic carcinogenesis and co-carcinogenesis to be conducted in species that approximate humans in their ability to metabolize arsenic (i.e., rabbits, hamsters and mice, in that order). Similarly, *in vitro* studies of co-mutagenesis or other mechanistically relevant endpoints should employ human epithelial cells (primary cultures when possible) rather than cell lines of mesenchymal origin or immortalized or tumor-derived cell lines. Greater attention to species and cell type selection would minimize problems related to the interpretation of results obtained in tissues that differ in important ways from normal human epithelium, which appears to be the most common target for arsenic carcinogenesis.

References

Albores, A.; Koropatnick, J.; Cherian, M.G.; and Zelazowski, A.J. (1992) Arsenic induces and enhances rat hepatic metallothionein production *in vivo*. Chem.-Biol. Interactions 85: 127-140.

Aposhian, H.V. (1989) Biochemical toxicology of arsenic. Rev. Biochem. Toxicol. 10: 265-299.

Aposhian, H.V. (1997) Enzymatic methylation of arsenic species and other new approaches to arsenic toxicity. Annu. Rev. Pharmacol. Toxicol. 37: 397-419.

Baylin, S.B.; Herman, J.G.; Graff, J.R.; Vertino, P.M.; and Issa, J.P.J. (1997) Alterations in DNA methylation: a fundamental aspect of neoplasia. Adv. Cancer Res.

Beck, B.D.; Boardman, P.D.; Hook, G.C.; Rudel, R.A.; Slayton, T.M.; and Carlson-Lynch, H. (1995) Response to Smith et al. (letter). Environ. Health Perspect. 103: 15-17.

Berg, J. (1990) Zinc finger domains: hypothesis and current knowledge. Ann. Rev. Biophys. Chem. 19: 405-421.

Buchet, J.P.; Lauwerys, R.; and Roels, H. (1981a). Comparison of the urinary excretion of arsenic metabolites after a single dose of sodium arsenite, monomethylarsonate or dimethylarsinate in man. Int. Arch. Occup. Environ. Health 48: 71-79.

Buchet, J.P.,;Lauwerys, R.; and Roels, H. (1981b) Urinary excretion of inorganic arsenic and its metabolites after repeated ingestion of sodium metaarsenite by volunteers. Int. Arch. Occup. Environ. Health 48: 111-118.

Buchet, J.P.; and Lauwerys, R. (1985) Study of inorganic arsenic methylation by rat liver *in vitro:* relevance for the interpretation of observations in man. Arch. Toxicol. 57: 125-129.

Carlson-Lynch H.; Beck, B.D.;, and Boardman, P.D. (1994) Arsenic risk assessment. Environ. Health Perspect. 102: 354-356.

Cerutti, P.A. (1985) Prooxidant states and tumor promotion. Science 227: 375-381.

Chiang, P.K.; Gordon, R.K.; Tal, J.; Zeng, G.C.; Doctor, B.P.; Pardhasaradhi, K.; and McCann, P.P. (1996) S-Adenosylmethionine and methylation. FASEB J. 10: 471-480.

Chiou, H.Y.; Hsueh, Y.M.; Liaw, K.F.,;Horng, S.F.; Chiang, M.H.; Pu, Y.S.; Lin, J.S.; Huang, C.H.; and Chen, C.J. (1995) Incidence of internal cancers and ingested inorganic arsenic: a seven-year follow-up study in Taiwan. Cancer Res. 55: 1296-1300.

Concha, G.; Nermell, B.; and Vahter, M. (submitted) Metabolism of inorganic arsenic in Argentine children in relation to exposure, gender, age and ethnic origin.

Costa, M. (1995) Model for the epigenetic mechanism of action of nongenotoxic carcinogens. Am. J. Clin. Nutr. 61 (Supp): 666S-669S.

Counts, J.L. and Goodman, J.I. (1995) Alterations in DNA methylation may play a variety of roles in carcinogenesis. Cell 83: 13-15.

Del Razo, L.M.; García-Vargas, G.; Vargas, H.; Albores, A.; and Cebrián, M. (1995) Chronic high arsenic exposure alters the profile of urinary arsenic metabolites in humans. Toxicologist 15: 262.

Dulout, F.N.; Grillo, C.A.; Sesane, A.I.; Maderna, C.R.; Nilsson, R.; Vahter, M.; Darroudi, F.; and Natarajan, A.T. (1996) Chromosomal aberrations in peripheral blood lymphocytes from native Andean women and children from northwestern Argentina exposed to arsenic in drinking water. Mutation Res. 370: 151-158.

Eden, S. and Cedar, H. (1994) Role of DNA methylation in the regulation of transcription. Curr. Opin. Genet. Dev. 4: 255-259.

Elhajouji, A.P.; van Hummelen, P.; and Kirsch-Volders, M. (1995) Indications for a threshold of chemically-induced aneuploidy *in vitro* in human lymphocytes. Environ. Mol. Mutagen. 26: 292-304.

Elia, M.C.; Storer, R.D.; McKelvey, T.W.; Kraynak, A.R.; Barnum, J.E.; Harmon, L.S.; DeLuca, J.G.; and Nichols, W.W. (1994) Rapid DNA degradation in primary rat hepatocytes treated with diverse cytotoxic chemicals: analysis by pulse field gel electrophoresis and implications for alkaline elution assays. Environ. Mol. Mutagen. 24: 181-191.

Goncharova, E.I.; and Rossman, T.G. (1995) The antimutagenic effects of metallothionein may involve free radical scavenging. In: Sarker, B. (ed.) Genetic Response to Metals (New York: Marcel Dekker, Inc.,): 87-100.

Goncharova, E.I.; Nidas, A.; and Rossman, T.G. (1996). Serum deprivation, but not inhibition of growth *per se,* induces a hypermutable state in Chinese hamster G12 cells. Cancer Res. 56: 752-756.

Healy, S.M.; Zakharyan, R.A.; and Aposhian, H.V. (1997) Enzymatic methylation of arsenic compounds IV: *in vitro* and *in vivo* deficiency of the methylation of arsenite and monomethylarsonic acid in the guinea pig. Mutation Res., in press.

Healy, S.M.; Casarez, E.A.; Ayala-Fierro; and Aposhian, H.V. (submitted) Arsenite methyltransferase activity in tissues of mice.

Hertz-Picciotto, I.; Smith, A.H., Holtzman, D.; Lipsett, M.; and Alexeeff, G. (1992) Synergism between occupational arsenic exposure and smoking in the induction of lung cancer. Epidemiology 3: 23-31.

Hopenhayn-Rich, C.; Smith, A.H.; and Goeden, H.M. (1993). Human studies do not support the methylation threshold hypothesis for the toxicity of inorganic arsenic. Environ. Res. 60: 161-177.

Hopenhayn-Rich C.; Biggs, M.L.; Smith, A.H.; Kalman, D.A.; and Moore, L.E. (1996) Methylation study of a population environmentally exposed to arsenic in drinking water. Environ. Health Perspect. 104: 620-628.

IARC (1987) IARC Monographs on the Evaluation of Carcinogenic Risks to Humans-Genetic and Related Effects: An Updating of IARC Monographs Vol. 1-42, Supplement 6, Lyon, 71-76.

Issa, J.P.J.; Baylin, S.B.; and Belinsky, S.A. (1996) Methylation of the estrogen receptor CpG island in lung tumors is related to the specific type of carcinogen exposure. Cancer Res. 56: 3655-3658.

Issa, J.P.J.; Baylin, S.B.; and Herman, J.G. (1997) DNA methylation changes in hematologic malignancies: biologic and clinical implications. Leukemia 11 (Supp. 1): S7-S11.

Jacobson-Kram, D.; and Montalbano, D. (1985) The reproductive effects assessment group's report on the mutagenicity of inorganic arsenic. Environ. Mutagen. 7: 787-804.

Jha, A.N.; Noditi, M.; Nilsson, R.; and Natarajan, A.T. (1992) Genotoxic effects of sodium arsenite on human cells. Mutation Res. 284: 215-221.

Joenje, H. (1989) Genetic toxicology of oxygen. Mutat. Res. 219: 193-208.

Jones, P.A. (1996) DNA methylation errors and cancer. Cancer Res. 56: 2463-2467.

Keyse, S.M.; and Tyrrell, R.M. (1989) Heme oxygenase is the major 32-kDa stress protein induced in human skin fibroblasts by UVA radiation, hydrogen peroxide, and sodium arsenite. Proc. Natl. Acad. Sci. USA 85: 99-103.

Klein, C.B.; and Rossman, T.G. (1990) Transgenic Chinese hamster V79 cell lines which exhibit variable levels of gpt mutagenesis. Environ. Mol. Mutagen. 16: 1-12.

Klein-Szanto, A.J.P.; and Slaga, T.J. (1982) Effects of peroxides on rodent skin: epidermal hyperplasia and tumor promotion. J. Invest. Dermatol. 79: 30-34.

Landolph, J.R. (1994). Molecular mechanisms of transformation of C3H/10T1/2 C1 8 mouse embryo cells and diploid human fibroblasts by carcinogenic metal compounds. Environ. Health Perspect. 102: 119-125.

Larramendy, M.L.; Popescu, N.C.; and DiPaolo, J.A. (1981) Induction by inorganic metal salts of sister-chromatid exchanges and chromosome aberrations in human and in Syrian hamster cell strains. Environ. Mutagen. 3: 597-606.

Lee, T.-C.; Huang, R.Y.; and Jan, K.Y. (1985b). Sodium arsenite enhances the cytotoxicity, clastogenicity and 6-thioguanine-resistant mutagenicity of ultraviolet light in Chinese hamster ovary cells. Mutat. Res. 148: 83-89

Lee, T.-C.; Oshimura, M.; and Barrett, J.C. (1985a). Comparison of arsenic-induced cell transformation, cytotoxicity, mutation and cytogenetic effects in Syrian hamster embryo cells in culture. Carcinogenesis 6: 1421-1426.

Lee, T.-C.; Lee, K.C.; Tzeng, Y.J.; Huang, R.Y.; and Jan, K.Y. (1986) Sodium arsenite potentiates the clastogenicity and mutagenicity of DNA crosslinking agents. Environ. Mutagen. 8: 119-128.

Lee, T.-C.; and Ho, I.C. (1994) Differential cytotoxic effects of arsenic on human and animal cells. Environ. Health Perspect. 3: 101-105.

Lee, Y.W.; Klein, C.B.; Kargacin, B.; Salnikow, K.; Kitahara, J.; Dowjat, K.; Zhitkovich, A.; Christie, N.T.; and Costa, M. (1995) Carcinogenic nickel silences gene expression by chromatin condensation and DNA methylation: a new model for epigenetic carcinogens. Mol. Cell Biol. 15: 2547-2557.

Lee-Chen, S.F.; Yu, C.T.; and Jan, K.Y. (1992) Effect of arsenite on the DNA repair of UV-irradiated Chinese hamster ovary cells. Mutagenesis 7: 51-55.

Lee-Chen, S.F.; Yu, C.T.; Wu, D.R.; and Jan, K.Y. (1994) Differential effects of luminol, nickel, and arsenite on the rejoining of ultraviolet light and alkylation-induced DNA breaks. Environ. Mol. Mutagenesis 23: 116-120.

Lèonard, A.;and Lauwerys, R.R. (1980) Carcinogenicity, teratogenicity and mutagenicity of arsenic. Mutat. Res. 75: 49-62.

Lerda, D. (1994) Sister-chromatid exchange (SCE) among individuals chronically exposed to arsenic in drinking water. Mutati. Res. 312: 111-120.

Lever, W.F.; and Schaumburg-Lever, G. (1983) Histopathology of the Skin, 6th edition (Philadelphia: J.B. Lippenett Company): 267-268.

Lewis, J.D.; Meehan, R.R.; Henzel, W.J.; Maurer-Fogy, I.; Jeppesen, P.,;Klein, F.; and Bird, A. (1992) Purification, sequence, and cellular localization of a novel chromosomal protein that binds to methylated DNA. Cell 69: 905-914.

Li, J.-H. (1989) Ph.D. Thesis, New York University.

Li, J.-H.; and Rossman, T.G. (1989a) Mechanism of comutagenesis of sodium arsenite with N-methyl-N-nitrosourea. Biol. Trace Element Res. 21: 373-381.

Li, J.-H.; and Rossman, T.G. (1989b) Inhibition of DNA ligase activity by arsenite: a possible mechanism of its comutagenesis. Mol. Toxicol. 2: 1-9.

Li, J.-H.; and Rossman, T.G. (1991) Comutagenesis of sodium arsenite with ultraviolet radiation in Chinese hamster V79 cells. Biol. Metals 4: 197-200.

Little, J.B. (1994) Failla memorial lecture: changing views of radiosensitivity. Radiat. Res. 140: 299-236.

Livingstone, L.R.; White, A.; Sprouse, J.; Livanos, E.; Jacks, T.; and Tisty, T.D. (1992) Altered cell cycle arrest and gene amplification potential accompany loss of wild-type p53. Cell 70: 923-935.

Mappes, R. (1977) Experiments on excretion of arsenic in urine. Internat. Arch. Occup. Environ. Health 40: 267-272.

Mass, M.J.; and Wang, L. (1997) Arsenic alters cytosine methylation patterns of the promoter of the tumor suppressor gene in human lung cells: a model for mechanism of carcinogenesis. Mutat.Res. 386: 263-277.

Meehan, R.; Lewis, J.; Cross, S.; Nan, X.; Jeppesen, P.; and Bird, A. (1992) Transcriptional repression by methylation of CpG. J. Cell Sci. (Supp.) 16: 9-14.

Meng, Z.; and Hsie, A.W. (1996) Polymerase chain reaction-based deletion analysis of spontaneous and arsenite-enhanced gpt mutants in CHO-AS52 cells. Mutat. Res. 356: 255-0259.

Murai, T.; Iwata, H.; Otoshi, T.; Endo, G.; Horiguchi, S.; and Fukushima, S. (1993) Renal lesions induced in F344/DuCrj rats by 4-weeks oral administration of dimethylarsinic acid. Tox. Letters 66: 53-61.

Mushak, P.; and Crocetti, A.F. (1996) Response: accuracy, arsenic and cancer. Environ. Health Perspect. 104: 1014-1018.

National Research Council (1977) Medical and Biologic Effects of Environmental Pollutants, Arsenic. (Washington, D.C.: National Academy of Sciences).

Nordenson, I.; and Beckman, L. (1991) Is the genotoxic effect of arsenic mediated by oxygen free radicals? Hum. Hered. 41: 71-73.

Nordenson, I.; Beckman, G.; Beckman, L.; and Nordstrom, S. (1978) Occupational and environmental risks in and around a smelter in Northern Sweden. II. Chromosomal aberrations in workers exposed to arsenic. Hereditas 88: 47-50.

Okui, T.; and Fujiwara, Y. (1986) Inhibition of human excision DNA repair by inorganic arsenic and the comutagenic effect in V79 Chinese hamster cells. Mutat. Res. 172: 69-76.

Oya-Ohta, Y.; Kaise, T.; and Ochi, T. (1996) Induction of chromosomal aberrations in cultured human fibroblasts by inorganic and organic arsenic compounds and the different roles of glutathione in such induction, Mutat. Res. 357: 123-129.

Preston, R.J. (1991) Mechanisms of induction of chromosomal alterations and sister chromatid exchanges: presentation of a generalized hypothesis. In Li, A.P., and Heflich, R.F. (eds.) Genetic Toxicology: A Treatise (New Jersey: Telford Press): 41-66.

Pryor, W.A. (1986) Cancer and free radicals. Basic Life Sci. 39: 45-59.

Ramos, O.; Carrizales, L.; Yanez, L.; Mejia, J.; Batres, L.; Ortiz, D.; and Diaz-Barriga, F. (1995) Arsenic increased lipid peroxidation in rat tissues by a mechanism independent of glutathione levels. Environ. Health Perspect. 103: 85-88.

Rasmussen, R.E.; and Menzel, D.B. (1997) Variation in arsenic-induced sister chromatid exchange in human lymphocytes and lymphoblastoid cell lines. Mutat.Res. 386: 299-306.

Rossman, T.G. (1981) Enhancement of UV-mutagenesis by low concentrations of arsenite in *E. coli.* Mutat. Res. 91: 207-211.

Rossman, T.G. (1994) Metal mutagenesis. In Goyer, R.A. and Cherian, M.G. (eds.) Toxicology of Metals: Biochemical Aspects. (New York: Springer-Verlag): 374-405.

Rossman, T. G. (1997) Molecular and Genetic Toxicology of Arsenic. In Rose, J. (ed.) Environmental Toxicology. (Gordon and Breach Publishers): (in press).

Rossman, T.G.; Molina, M.; and Meyer, L.W. (1984) The genetic toxicology of metal compounds I: Induction of l prophage in *E. coli* WP2s(l). Environ. Mutagen. 6: 59-69.

Rossman, T.G.; Goncharova, E.I.,;Rajah, T.; and Wang, Z. (1997) Human cells lack the inducible tolerance to arsenite seen in Chinese hamster cells. Mutat. Res. (in press).

Rossman, T.G.; Stone, D.; Molina, M.; and Troll, W. (1980). Absence of arsenite mutagenicity in *E. coli* and Chinese hamster cells. Environ. Mutagen. 2: 371-379.

Rossman, T.G.; and Wolosin, D. (1992) Differential susceptibility to carcinogen-induced amplification of SV40 and dhfr sequences in SV40-transformed human keratinocytes. Mol. Carcinogen. 6: 203-213.

Rudel, R.,;Slayton, T.M.; and Beck, B.D. (1996) Implications of arsenic genotoxicity for dose response of carcinogenic effects. Reg. Toxicol. Pharm. 23: 87-105.

Saffiotti, U.; and F. Bertolero (1989). Neoplastic transformation of BALB/3T3 cells by metals and the quest for induction of a metastatic phenotype. Biol. Trace Element Res. 21: 475-482.

Scott, N.; Hatlelid, K.M.,;MacKenzie, N.E.; and Carter, D.E. (1993) Reactions of arsenic (III) and arsenic (V) species with glutathione. Chem. Res. Toxicol. 6: 102-106.

Slaga, T.J.; Klein-Szanto, A.J.P.; Triplett, L.; Yotti, L; and Trosko, J.E. (1981) Skin tumor promoting activity of benzoyl peroxide, a widely used free radical generating compound. Science 213: 1023-1025.

Snyder, R.D.; Davis, G.F.; and Lachmann, P. (1989) Inhibition by metals of x-ray and ultraviolet-induced DNA repair in human cells. Biol. Trace Element Res. 21: 389-398.

Soderhall, A.; and Lindhahl, T. (1987) DNA ligase of eukaryotes. FEBS Lett. 67: 1-8.

Styblo, M.; Serves, S.V.; Cullen, W.R.; and Thomas, D.J. (1997) Comparative inhibition of yeast glutathione reductase by arsenicals and arsenothiols. Chem. Res. Toxicol. 10: 27-33.

Tam, G.K.H.; Charbonneau, S.M.; Bryce, F.; Pomroy, C.; and Sandi, E. (1979) Metabolism of inorganic arsenic (^{74}As) in humans following oral ingestion. Toxicol. Appl. Pharmacol. 50: 319-322.

Tinwell, H.; Stephens, S.C.; and Ashby, J. (1991) Arsenite as the probable active species in the human carcinogenicity of arsenic: mouse micronucleus assays on Na and K arsenite, orpiment, and Fowler's solution. Environ. Health Perspect. 95: 205-210.

Tkeshelashvili, L.K.; Shearman, C.W.; Zakour, R.A.; Koplitz, R.M.; and Loeb, L.A. (1980) Effects of arsenic, selenium, and chromium on the fidelity of DNA synthesis. Cancer Res. 40: 2455-2460.

U.S. EPA (1996) Proposed guidelines for carcinogen risk assessment; notice. Fed. Reg. 61: 17960-18011 (April 23, 1996).

Vahter, M. (1983) Environmental and occupational exposure to inorganic arsenic. Acta Pharmacol. Toxicol. 59 (Supp 11): 31-34.

Vahter, M.; and Marafante, E. (1989) Intracellular distribution and chemical forms of arsenic in rabbits exposed to arsenate. Biol. Trace Elem. Res. 21: 233-239.

Vahter, M.; Concha, G.; Nermell, B.; Nilsson, R.; Duluot, F.; and Natarajan, A. (1995) A unique metabolism of inorganic arsenic in native Andean women. Eur. J. Pharmacol. 293: 455-462.

Vega, L.; Gonsebatt, M.E.; and Ostrosky-Wegman, P. (1995) Aneugenic effect of sodium arsenite on human lymphocytes *in vitro:* an individual susceptibility effect detected. Mutation Res. 334: 365-373.

Vig, B.K.; Figueroa, M.L.; Cornforth, M.N.; and Jenkins, S.H. (1984) Chromosome studies in human subjects chronically exposed to arsenic in drinking water. Am. J. Ind. Med. 6: 325-338.

Wang, T.S.; and Huang, H. (1994) Active oxygen species are involved in the induction of micronuclei by arsenite in XRS-5 cells. Mutagenesis 9: 253-257.

Wang, T.S.; Kuo, C., Jan, K.; and Huang, H. (1996) Arsenite induces apoptosis in Chinese hamster ovary cells by generation of reactive oxygen species. J. Cell Physiol. 169: 256-268.

Wang, Z.; and T.G. Rossman (1996) The carcinogenicity of arsenic. In Chang, L.W. (ed.) Toxicology of Metals (Boca Raton: CRC Press): 219-227.

Wang, Z.; Dey, S.; Rosen, B.P.; and Rossman, T.G. (1996) Efflux-mediated resistance to arsenicals in arsenic-resistant and -hypersensitive Chinese hamster cells. Toxicol. Appl. Pharmacol. 137: 112-119.

Wanibuchi, H.; Yamamoto, S.; Chen, H.; Yoshida, K.; Endo, G.,;Hori, T.; and Fukushima, S. (1996) Promoting effects of dimethylarsinic acid on N-butyl-N-(4-hydroxybutyl)nitrosamine-induced urinary bladder carcinogenesis in rats. Carcinogenesis 17: 2435-2439.

Wanibuchi, H., et al. (unpublished) Promotion of rat hepatocarcinogenesis by dimethylarsinic acid: association with elevated ornithine decarboxylase activity and formation of 8-hydroxydeoxyguanosine in the liver.

Warner, M.L.; Moore, L.E.; Smith, M.T.; Kalman, D.A.; Fanning, E.; and Smith, A.H. (1994) Increased micronuclei in exfoliated bladder cells of individuals who chronically ingest arsenic-contaminated water in Nevada. Cancer Epidemiol. Biomarkers Prev. 3: 583-590.

Wiencke, J.K.; and Yager, J.W. (1992) Specificity of arsenite in potentiating cytogenetic damage induced by the DNA crosslinking agent diepoxybutane. Environ. Mol. Mutagen. 19: 195-200.

Wiltse, J.; and Dellarco, V. (1996) U.S. Environmental Protection Agency guidelines for carcinogen risk assessment: past and future. Mutat. Res. 365: 3-15.

Xuan, X.Z.; Lubin, J.H.; Li, J.Y.; Yang, L.F.; Luo, A.S.; Lan, Y.; Wang, J.Z.; and Blot, W.J. (1993) A cohort study in southern China of tin miners exposed to radon and radon decay products. Health. Phys. 64: 120-131.

Yamamoto, S.; Konishi, Y.; Matsuda, T.; Murai, T.; Shibata, M.A.; Matsui-Yuasa, I.; Otani, S.; Kuroda, K.; Endo, G.; and Fukushima, S. (1995) Cancer induction by an organic arsenic compound, dimethylarsinic acid (cacodylic acid), in F344/DuCrj rats after pretreatment with five carcinogens. Cancer Res. 55: 1271-1276.

Yamanaka, K.; Ohba, H.; Hasegawa, A.; Sawamura, R; and Okada, S. (1989) Mutagenicity of dimethylated metabolites of inorganic arsenic. Chem. Pharm. Bull. 37: 2753-2756.

Yamanaka, K.; Hoshino, M.; Okamoto, M.; Sawamura, R.; Hasegawa, A.; and Okada, S. (1990) Induction of DNA damage by dimethylarsine, a metabolite of inorganic arsenics, is for the major part likely due to its peroxyl radical. Biochem. Biophys. Res. Commun. 168: 58-64.

Yamanaka, K; and Okada, S. (1994) Induction of lung-specific DNA damage by metabolically methylated arsenics via the production of free radicals. Environ. Health Perspect. 102: 37-40.

Yamanaka, K.; Ohtsubo, K.; Hasegawa, A.; Hayashi, H.; Ohji, H.; Kanisawa, M.; and Okada, S. (1996) Exposure to dimethylarsinic acid, a main metabolite of inorganic arsenics, strongly promotes tumorigenesis initiated by 4-nitroquinoline 1-oxide in the lungs of mice. Carcinogenesis 17: 767-770.

Yang, J.-L.; Chen, M.-F.; Wu, C.-W.; and Lee, T.-C.; (1992) Posttreatment with sodium arsenite alters the mutation spectrum induced by ultraviolet light irradiation in Chinese hamster ovary cells. Environ. Mol. Mutagen. 20: 156-164.

Zakharyan, R.A.; Wu, Y.; Bogdan, G.M.; and Aposhian, H.V. (1995) Enzymatic methylation of arsenic compounds I: assay, partial purification, and properties of arsenite methyltransferase and monomethylarsonic acid methyltransferase of rabbit liver. Chem. Res. Toxicol. 8: 1029-1038.

Zakharyan, R.A.; Wildfang, E.; and Aposhian, H.V. (1996) Enzymatic methylation of arsenic compounds III: the marmoset and tamarin, but not the rhesus, monkey are deficient in methyltransferases that methylate inorganic arsenic. Toxicol. Appl. Pharmacol. 140: 77-84.

V. OBSERVER COMMENTS

At several different points during the meeting, Dr. Preston opened the floor to comments from the observers attending the workshop. The observations and suggestions offered during these comment periods are summarized below, along with any responses from members of the expert panel.

George Parris, AWPI

Noting that arsenic occurs in many different types of molecules and that these different forms may have different effects, this commenter urged greater attention to issues related to both the qualitative and quantitative differences among the various species. From a regulatory perspective, he thought it would also be important to make a clear distinction among the effects associated with different routes of exposure.

Dr. Aposhian agreed that it is important to distinguish among the various species as much as possible. In response to the comment about route of administration, he noted that the focus of the workshop on drinking water implied a focus on oral exposures. That said, however, he expressed the view that inhalation exposure is likely to become a much more serious problem as emissions from smelters in Mexico begin to cause health problems in the southern United States. He also commented on the sharp contrast between the cooperation evidenced by the waterworks industry and the lack of cooperation from the mining and smelter industries, which have historically been reluctant to cooperate with researchers attempting to sort out the health effects of arsenic.

Marc Mass, EPA Health and Environmental Effects Research Laboratory

After noting that his comments do not reflect EPA policy, this observer remarked on the conspicuous lack of attention throughout the panel's discussions to the lung as a target tissue for arsenic, particularly since the IARC classification of arsenic as a carcinogen is based on the incidence of lung cancer in smelter workers.

Regarding a reference to his own work that had been made during the discussion of arsenic's mode of action, the commenter noted that although his group has demonstrated hypermethylation of DNA induced by arsenic, another laboratory (results unpublished) has shown hypomethylation. Because of this, he suggested that it might be more accurate to describe arsenic as an agent that changes DNA methylation patterns rather than as a hypermethylating agent. The observer also thought that in its discussion of replication fidelity, the panel should consider the work of Larry Loeb, who has demonstrated that neither arsenite nor arsenate has an effect on DNA replication in isolated systems.

With respect to questions of arsenic speciation, the commenter suggested that it is important to consider the implications if some or all of the carcinogenic activity of arsenic is actually due to the methylated metabolites. If this were true, it might mean that responses to arsenic could be supra-linear at low doses, since that is the region of the dose-response curve where metabolism would presumably be most efficient. Likewise, risk would tend to increase with methylation efficiency, rather than decrease, as would be the case if methylation functions as a detoxification mechanism.

In response to this comment, Dr. Aposhian noted that his group has been very interested in studying tumor incidence rates in the subset of animals that metabolize arsenic by processes other than methylation (e.g., marmoset monkeys and chimpanzees). In addition, he thought it important to keep in the mind the large variation in rates of arsenic metabolism in humans, which may suggest some sort of polymorphism.

Dr. Rossman pointed out that in at least one study arsenite has been shown to be more active than MMA or DMA, by several orders of magnitude, in producing chromosomal aberrations. Dr. Park noted that while supra-linearity is common in the observable dose range, it has never, to his knowledge, been demonstrated at low doses; the reason for this, he speculated, is that supra-linearity usually involves saturation of some sort, which is by definition a high-dose phenomenon. Dr. Issa suggested that it would be difficult to establish a role for hypomethylation in tumorogenesis, since hypomethylation is a very generalized phenomenon in tumors and is also a normal physiologic response to proliferation.

Howard Greene, ARCO

The commenter began by noting that he represents the Environmental Arsenic Council, a group of companies interested in promoting the use of sound science in the development of a new and improved arsenic risk assessment. He indicated that the Council agrees with the panel's conclusion that there is a sufficient body of evidence to support the use of a nonlinear or threshold model in describing the relationship between arsenic and skin cancer and that there is no evidence that arsenic acts as a direct carcinogen. Regardless of the model adopted, however, the Council believes that the risk assessment should not be based on the Taiwanese data, due to the poor quality of both exposure estimates and prevalence data in the Tseng study. The commenter also said that the Council urges the expert panel to encourage EPA to finalize the arsenic risk assessment and to include a range of risk extrapolations rather than a single value for arsenic within the IRIS database; in addition to more accurately reflecting the current state of knowledge about arsenic carcinogenicity, a range of values might also give risk managers a better appreciation of the amount of uncertainty in the risk assessment.

Dr. Preston thanked the observer for his comments, but noted that the charge to the panel wasto review the available literature and determine what conclusions could plausibly be drawn about a mode of action for arsenic. Although he indicated that it might be appropriate for the panel to suggest ways of reducing the uncertainty associated with these conclusions, he thought that the types of recommendations suggested by the observer would be outside the Agency's charge to the panel.

Saskia Mooney, Weinberg, Bergeson & Neuman

This observer stated that she had been sent to the meeting specifically to ask Dr. Rossman whether the results of recent epidemiologic studies could be explained by her work showing a lack of immunologic responses to arsenic in keratinocytes. Dr. Rossman indicated that her work did not address immunologic responses. Further, she felt that it would be quite a stretch to attempt to explain the results of human epidemiologic studies based on the kinds of work she has been doing in the laboratory. A more fruitful line of inquiry, she suggested, would be to take lymphocytes or fibroblasts from arsenic-exposed individuals and see whether those have or have not become tolerant to the effects of arsenic.

David Craigin, ELF Atochem North America

While recognizing that the charge to the panel was to advise EPA regarding a plausible mode of action for arsenic, this observer suggested that the panel emphasize to the Agency the importance of a risk assessment that is relevant to the real world. Presently, he noted, the MCL for arsenic is 2,777 times higher than the risk-based Clean Water Act criterion; as a result, calculated criterion levels for arsenic are typically 10- to 100-fold below natural background levels, and target levels for soil and water often turn out to be far lower than normal dietary exposures to arsenic. As a step toward correcting this problem, the commenter urged the panel to direct EPA's attention to studies suggesting that arsenic is a required nutrient in at least some species, and to take background exposure levels into consideration when thinking about the possibility of a threshold in the dose-response curve.

In response to this comment, Dr. Aposhian said that he thought it very important for EPA and other federal agencies to be more cognizant of ways in which their efforts do or do not help suffering humans; based on his own experience, he worries that EPA and other agencies sometimes get more focused on meeting a paper-based criterion cleanup level than on assuring that the very real health needs of exposed individuals are adequately addressed.

Charles Abernathy, EPA Office of Water

As an example of inter-individual variation in responses to arsenic, this commenter described the results of a case in Latin America in which only one member of a family exposed to arsenical pesticides exhibited any symptoms. Further investigation indicated that the affected individual was deficient in 5,10-methylene-tetrahydrofolate reductase, an enzyme involved in the methylation reactions.

Regarding the issue of dietary arsenic intake, the commenter pointed out that arsenic in the diet occurs mostly in the form of organic arsenicals. According to the FDA food basket survey, human dietary intake of arsenic is roughly 50 μg/day, but only about 10 to 15 μg of this is inorganic arsenic. Although inorganic arsenicals are occasionally methylated by fish and shellfish, they are much more commonly metabolized to arsenocholine or arsenobetane derivatives, which are far less reactive and far less toxic than the methylated metabolites of inorganic arsenic.

Commenting on the epidemiologic studies that have been conducted to date, this observer noted that what all of the U.S. studies have in common is their lack of statistical power. In the Utah study, for example, it was not possible to determine whether the observed difference in nerve conduction velocity was significant, as the study was too small.

Other problems plague interpretation of the Tseng study. Among these is the researchers' use of the Natelson method for analyzing arsenic levels, which fails to pick up some species altogether. Moreover, the Natelson method has a detection limit of 30 μg/L and a quantification limit of 80 μg/L; in spite of these methodologic limits, however, the data include many numbers in the 10 to 30 μg/L range.

Yung-Pin Liu, National Cancer Institute

After noting that the National Cancer Institute's only 1997 grant for arsenic-related work was awarded to panelist Toby Rossman, this observer announced that the Institute is planning a 3-day workshop on Arsenic Health and Research Issues, which has tentatively been scheduled for September 22 to 24, 1997. This meeting, which is being co-sponsored by the National Institute for Environmental Health Sciences and by EPA, will take a more research-oriented look at some of the questions that remain about arsenic's mechanism of carcinogenic action.

Roseanne Lorenzana, EPA Region X

Noting that EPA Region X has been directed to perform site-specific risk assessments for tribal villages in Alaska that are using groundwater with elevated arsenic levels as a drinking water source, this observer asked the panel to comment on particular measures of both exposure and response that should be monitored in these populations.

Dr. Aposhian responded that by using a technique such as DMPS, it is now possible to get a much better idea of the body burden of arsenic than was previously possible. Dr. Park encouraged as much reliance as possible on direct measures of exposure, and as little as possible on modeling. Dr. Issa commented that it might be interesting to look at chromosomal anomalies in shed cells, but Dr. Preston said that this would make sense only if there were reliable techniques for correlating dose with exposure. Dr. Klein-Szanto thought that it would also be important to establish what exposure individuals or the study population as a whole may have had to other putative carcinogens.

William Marcus, EPA Office of Water

This observer described the results of two recent studies of arsenic conducted in the United States. One of these studies, conducted near a Baltimore plant that produces organic arsenicals, used zip code and meteorologic considerations as a surrogate for exposure; in this study, there was an increase in arsenic load and a decrease in nerve conduction velocity in children. A second study, conducted near a plant in Washington state and using a nearby aluminum production facility as a control, also found decreases in nerve conduction velocity, particularly in children.

To enhance the statistical power of epidemiologic studies, this observer recommended stratification of the study population by methylation potential. Against such a background, he thought that it would probably be possible to pick up significant effects, at least in places such as Alaska, where exposure levels are unusually high. In addition, however, it is important to control for sufficient protein and vitamin consumption in these populations.

Daniel Byrd, Inner Mongolian Cooperative Arsenic Project

This commenter began by noting that the project he represents began in the early 1990s, when he and his colleagues conducted studies in a number of different arsenic-exposed populations and found that the tumor incidence data in these populations were in both qualitative and quantitative agreement with the results of the Taiwanese study. Noting that the results of these studies have been published, he questioned why these data were not included in the material reviewed by the expert panelists. In recent years, the group has focused on a population in Inner Mongolia; epidemiologic results from this population have been published in the Chinese literature and translations are beginning to reach the English-speaking press.

In addition to the Inner Mongolian studies, this observer thought that the panel should be aware of a re-analysis of the Taiwanese cohort conducted by Ken Brown and C.J. Chen. Although there remain problems with the exposure data, the response data have now been sorted out almost to the level of individuals in the villages. Given his understanding that dose-response curves are empirical rather than theoretical entities, the observer suggested that the panel consider these studies as part of their deliberations. In response to Dr. Aposhian's request, the observer agreed to provide copies of the papers he had described to the panel.

Gary Carlson, Purdue University

Noting that many of the initiation/promotion and *in vitro* studies for arsenic were done using DMA, this commenter wondered how the panel's confidence in any particular mode of action addresses the uncertainty as to which arsenic species is really the "bad actor." Presumably, if DMA is the main carcinogen, methylation would be considered an intoxication process; if DMA exerts the same sort of protective effect for carcinogenesis that it is believed to exert in the setting of noncancer endpoints, however, methylation would represent a detoxification.

Dr. Rossman observed that the high concentrations of DMA used in many animal and cell culture experiments may be of little real relevance, since these concentrations are unlikely ever to be reached *in vivo*. If arsenite is toxic at 10 millimolar, it really doesn't matter that DMA isn't toxic until 100 millimolar, unless the exposure were to DMA itself. She also disagreed that DMA has been shown to be the "bad actor" in cell culture studies, suggesting that a peroxy radical actually causes the damage.

Dr. Aposhian added that it has only been in the last year or so that toxicologists have begun to question whether the methylation of arsenic is really a detoxification reaction. That there are numerous species that do not methylate arsenic suggests to him that methylation is certainly not the only, and may not even be the primary, detoxification pathway. Although it is so far difficult to demonstrate, his group is currently exploring protein binding as an alternative pathway for arsenic detoxification.

Harvey Clewell, ICF Kaiser

This observer commended the panel for its thoughtful approach to the question of linearity versus nonlinearity in the dose-response curve for arsenic, noting that epidemiologists seem to have believed for some time that the curve is nonlinear, but toxicologists haven't gotten very far in their efforts to explain why this might be. Because of the panel's deliberations, this observer thought that it would be much easier for EPA to decide on an appropriate margin of safety for drinking water despite knowing only that tumors are likely to occur at some much higher level of exposure.

Arnold Kuzmack, EPA Office of Water

This observer noted that an appropriate margin of exposure for arsenic would also need to take into account the difference between skin tumors, which pose relatively little risk to life, and internal tumors, where the risk is greater. In addition to clarifying the mode of action for arsenic, therefore, it will also be important to determine what the weight of evidence is for arsenic as a carcinogen in the setting of internal cancers.

Bernard Wagner, Wagner Associates, Inc.

This commenter pointed out that the Armed Forces Institute of Pathology maintains an extensive registry of pathology that goes back to the mid-1800s, and he suggested that this data might be useful in looking at whether there is any correlation between the distribution of tumors in the United States and the areas where arsenic is elevated in drinking water. Noting that he had not attended the first day of the workshop, the commenter also asked whether the panel did or did not consider low-dose arsenic in drinking water a human carcinogen. Dr. Cohen, to whom the question was addressed, responded that in concluding that the dose-response curve is probably nonlinear, the panel is also concluding that there is some low dose at which arsenic is probably safe. The problem, however, is that neither the panel nor anyone else knows what that dose is.

Paul White, EPA Office of Research and Development

Noting that the panel seems to agree that much remains unknown about the mechanism of arsenic carcinogenicity, this observer expressed surprise at the relative ease with which the panel was able to conclude that the dose-response curve is nonlinear and may involve a threshold. One reason for his concern has to do with diversity in the human population, and another with the possibility that arsenic may act as a co-carcinogen. In view of the statistical literature suggesting that compounds that add on to an ongoing process of carcinogenesis tend to have linear dose-response curves in the low-dose region, this observer urged the panel to think more about the additivity issue. Dr. Preston responded that the charge to the panel was to determine whether what is known about arsenic's mode of action can be used to predict the general shape of the dose-response curve; although the risk to specific populations is certainly something that should be considered by risk assessors, the question of sensitive populations was not among the issues the panel was asked to address. In addition, a form of nonlinear dose-response curve has an effectively linear slope at very low exposure levels, which would be consistent with the observer's comments.

Appendix A

MEETING ATTENDEES

United States
Environmental Protection Agency
National Center for Environmental Assessment

Expert Panel on Arsenic Carcinogenicity: Review and Workshop

Holiday Inn—National Airport
Washington, DC
May 21-22, 1997

List of Panel Members

Vasken Aposhian
University of Arizona
Life Sciences Building
P.O. Box 210106
Tucson, AZ 85721-0106
520-621-7565
Fax: 520-621-3709
E-mail: vas_aposhian@tikal.biosci.arizona.edu

Samuel Cohen
University of Nebraska Medical Center
Pathology/Microbiology Department
600 South 42nd Street
Omaha, NE 68198-3135
402-559-6388
Fax: 402-559-9297

Jean-Pierre Issa
Johns Hopkins Oncology Center
424 North Bond Street
Baltimore, MD 21231
410-955-8506
Fax: 410-614-9884

Andres Klein-Szanto
Department of Pathology
Fox Chase Cancer Center
7701 Burholme Avenue
Philadelphia, PA 19111
215-728-3154
Fax: 215-728-2899

Colin Park
Health and Environmental Sciences
The Dow Chemical Company
1803 Building
Midland, MI 48674
517-636-1159
Fax: 517-638-2425

R. Julian Preston
Chemical Industry Institute of Toxicology
6 Davis Drive
Research Triangle Park, NC 27709
919-558-1367
Fax: 919-558-1300
E-mail: preston@ciit.org

Toby Rossman
Nelson Institute of Environmental Medicine
New York University Medical Center
Long Meadow Road
Tuxedo, NY 10987
914-351-2380
Fax: 914-351-3489
E-mail: rossman@charlotte.med.nyu.edu

Expert Panel on Arsenic Carcinogenicity: Review and Workshop

Holiday Inn—National Airport
Washington, DC
May 21-22, 1997

Final Observer List

Charles Abernathy
Toxicologist
Health and Ecological Criteria Division
Office of Water
U.S. Environmental Protection Agency
401 M Street, SW (4304)
Washington, DC 20460
202-260-5374
Fax: 202-260-1036
E-mail: abernathy.charles@epamail.epa.gov

Robert Beliles
Toxicologist
National Center for
Environmental Assessment
QUANT
U.S. Environmental Protection Agency
401 M Street, SW (8623)
Washington, DC 20460
202-260-3018
Fax: 202-260-3803
E-mail: beliles.robert@epamail.epa.gov

Robert Benson
Toxicologist
Pollution Prevention, State and Tribal
Assistance for Drinking Water
U.S. Environmental Protection Agency
999 18th Street (8P2W-MS)
Suite 500
Denver, CO 80202-2466
303-312-7070
Fax: 303-312-6131
E-mail: benson.bob@epamail.epa.gov

Michael Bolger
Branch Chief
Division of Programs and
Enforcement Policy
Contaminant Standards
Monintoring and Programs
Food and Drug Administration
200C Street, SW (HFS-308)
Washington, DC 20204
202-205-5234
Fax: 202-260-0498

Daniel Byrd
Principal
Inner Mongolian Cooperative
Arsenic Project
1225 New York Avenue, NW
Suite 150
Washington, DC 20005
202-371-0603
Fax: 202-484-6019
E-mail: ctraps@radix.net

Tara Cameron
Environmental Engineer
Standards and Risk
Management Division
Office of Ground Water
and Drinking Water
U.S. Environmental Protection Agency
401 M Street, SW (4607)
Washington, DC 20460
202-260-3702
Fax: 202-260-3762

Gary Carlson
Professor of Toxicology
and Associate Head
School of Health Sciences
Purdue University
1338 Civil Engineering Building
West Lafayette, IN 47907-1338
317-494-1419
Fax: 317-496-1377

Hillary Carpenter
Environmental Toxicologist
Environmental Health
Hazard Management
Minnesota Department of Health
121 East Seventh Place - Suite 220
P.O. Box 64975
St. Paul, MN 55164-0975
612-215-0928
Fax: 612-215-0975
E-mail: hillary.carpenter@
health.state.mn.us

Selene Chou
Environmental Health Scientist
Emergency Response & Scientific
Assessment Branch
Agency for Toxic Substances
and Disease Registry
1600 Clifton Road (E-29)
Atlanta, GA 30333
404-639-6308
Fax: 404-639-6324
E-mail: cjc3@cdc.gov

Harvey Clewell
Project Manager
ICF Kaiser
602 East Georgia Avenue
Ruston, LA 71270
318-242-5017
Fax: 318-255-4960

Kevin Connor
Staff Scientist
Karch and Associates
1701 K Street, NW - Suite 1000
Washington, DC 20006
202-463-0400
Fax: 202-463-0502

David Cragin
Senior Toxicologist/Risk
Assessment Specialist
ELF Atochem North America
2000 Market Street
Philadelphia, PA 19103
215-419-5880
Fax: 215-419-5800

John Davidson
Environmental Scientist
Multimedia Strategies Analysis Division
U.S. Environmental Protection Agency
401 M Street, SW (2123)
Washington, DC 20460
202-260-5483
Fax: 202-260-2300
E-mail: davidson.john@
epamail.epa.gov

Vicki Dellarco
Health and Ecological Criteria Division
Office of Water
U.S. Environmental Protection Agency
401 M Street, SW (4304)
Washington, DC 20460
202-260-7336
Fax: 202-260-1036

Joyce Donohue
Toxicologist
Health and Ecological Criteria Division
Office of Water
U.S. Environmental Protection Agency
401 M Street, SW (4304)
Washington, DC 20460
202-260-1318
Fax: 202-260-1036
E-mail: donohue.joyce@
epamail.epa.gov

Irene Dooley
Standards and Risk
Management Division
Office of Groundwater
and Drinking Water
U.S. Environmental Protection Agency
401 M Street, SW (4607)
Washington, DC 20460
202-260-9531
E-mail: dooley.irene@epamail.epa.gov

Julie Du
Toxicologist
Health and Ecological Criteria Division
Office of Water
U.S. Environmental Protection Agency
401 M Street, SW (4304)
Washington, DC 20460
202-260-7583
Fax: 202-260-1036

Robert Dyer
Acting Associate Director
National Center for
Environmental Assessment
U.S. Environmental Protection Agency
401 M Street, SW (8601)
Washington, DC 20460
202-260-2014
Fax: 202-260-0393
E-mail: dyer.robert@epamail.epa.gov

Michal Eldan
c/o Sherry Thaxton
Luxembourg - Pamol, Inc.
5100 Poplar Avenue - Suite 2746
Memphis, TN 38137
901-761-9475
Fax: 901-761-9477

Robert Fensterheim
Executive Director
Environmental Arsenic Council
1350 Eye Street, NW - Suite 200
Washington, DC 20005
202-962-9400
Fax: 202-289-3565
E-mail: rjf@regnet.com

James Garrison
Senior Project Scientist/Toxicologist
Woodward-Clyde
International - Americas
10975 El Monte - Suite 100
Overland Park, KS 66211
913-344-1024
Fax: 913-344-1011

Robert Goyer
Consultant
6405 Huntingridge Road
Chapel Hill, NC 27514
919-419-1804
Fax: 919-493-2174

Howard Greene
Senior Consultant
Corporate Health & Safety
ARCO
444 South Flower Street
Los Angeles, CA 90071
213-486-3643
Fax: 213-486-6402
E-mail: hgreene@is.arco.com

Peter Grevatt
Risk Assessment Coordinator
Division of
Environmental Protection
Strategic Planning and
Multimedia Programs Branch
U.S. Environmental Protection Agency
290 Broadway
New York, NY 10007-1866
212-637-3758
Fax: 212-637-3771

Lee Hofmann
Environmental Health Scientist
Office of Emergency
and Remedial Response
U.S. Environmental Protection Agency
401 M Street, SW (5202G)
Washington, DC 20460
703-603-8874
Fax: 703-603-9133
E-mail: hofmann.lee@epamail.epa.gov

Michael Hughes
Toxicologist
National Health and Environmental
Effects Research Laboratory
U.S. Environmental Protection Agency
(MD-74)
Research Triangle Park, NC 27711
919-541-2160
Fax: 919-541-5394

Jennifer Jinot
Environmental Health Scientist
Office of Research and Development
National Center for
Environmental Assessment
U.S. Environmental Protection Agency
401 M Street , SW (8623)
Washington, DC 20460
202-260-8913
Fax: 202-260-3803
E-mail: jinot.jennifer@epamail.epa.gov

Jeff Jones
Delta Analytical Corporation
619 Somersworth Way
Silver Spring, MD 20902
301-652-5495
Fax: 301-652-5408
E-mail: delta@delta-ac.com

Elaina Kenyon
Toxicologist
National Health and Environmental
Effects Research Laboratory
U.S. Environmental Protection Agency
(MD-74)
Research Triangle Park, NC 27711
919-541-0043
Fax: 919-541-5394
E-mail: kenyon.elaina@
epamail.epa.gov

Curtis Klaassen
Professor of Pharmacology
and Toxicology
Department of Pharmacology,
Toxicology, and Therapeutics
University of Kansas Medical Center
2108 Breidenthal Building
3901 Rainbow Boulevard
Kansas City, KS 66160-7417
913-588-7714
Fax: 913-588-7501

Arnold Kuzmack
Senior Science Advisor
Office of Water
U.S. Environmental Protection Agency
401 M Street, SW (4301)
Washington, DC 20460
202-260-5821
Fax: 202-260-5394
E-mail: kuzmack.arnold@
epamail.epa.gov

Yung-Pin Liu
Program Director
National Cancer Institute
6111 Executive Boulevard (7670)
Suite 551
Bethesda, MD 20892
301-496-5471

Roseanne Lorenzana
Toxicologist
Risk Evaluation Unit
Office of Environmental Assessment
U.S. Environmental Protection Agency
1200 Sixth Avenue (OFA-095)
Seattle, WA 98101
206-553-8002
Fax: 206-553-0119
E-mail: lorenzana.roseanne@
epamail.epa.gov

Amal Mahfouz
Senior Toxicologist
Health and Ecological Effects Division
Office of Water
U.S. Environmental Protection Agency
401 M Street, SW (4303)
Washington, DC 20460
202-260-9568
Fax: 202-260-1036
E-mail: mahfouz.amal@
epamail.epa.gov

William Marcus
Senior Science Advisor
Office of Science and Technology
Office of Water
U.S. Environmental Protection Agency
401 M Street, SW (4301)
Washington, DC 20460
202-260-7317

Reuben Mascarenhas
Toxicologist
National Center for
Environmental Toxicology
Water Research Center
Henley Road England
Medmenham, Marlo, Bucks SL7 2HD
01491-571531
Fax: 01491-579094
E-mail: mascarenhas@wrcplc.co.uk

Marc Mass
Research Scientist
National Health and Environmental
Effects Research Laboratory
U.S. Environmental Protection Agency
(MD-68)
Research Triangle Park, NC 27711
919-541-3514
Fax: 919-541-0694
E-mail: mass.marc@epamail.epa.gov

Robert McGaughy
Senior Scientist
National Center for
Environmental Assessment
U.S. Environmental Protection Agency
401 M Street, SW (8623)
Washington, DC 20460
202-260-5889
Fax: 202-260-3803
E-mail: mcgaughy.robert@
epamail.epa.gov

Margaret McVey
Program Officer
Board on Environmental
Studies and Toxicology
Committee on Toxicology
The National Research Council
2001 Wisconsin Avenue, NW (HA-354)
Washington, DC 20007
202-334-2545
Fax: 202-334-2752
E-mail: mmcvey@nas.edu

G. Wade Miller
President
Miller Management Group, Inc.
4746 North 40th Street
Arlington, VA 22207
703-536-7533
Fax: 703-536-7534
E-mail: wmiller483@aol.com

Amy Mills
Environmenal Scientist
National Center for
Environmental Assessment
U.S. Environmental Protection Agency
401 M Street, SW (8623)
Washington, DC 20460
202-260-0569
Fax: 202-260-3803
E-mail: mills.amy@epamail.epa.gov

Saskia Mooney
Environmental Analyst
Weinberg, Bergeson & Neuman
1300 I Street, NW - Suite 1000 W
Washington, DC 20005
202-962-8595
Fax: 202-962-8599
E-mail: smooney@wbnlaw.com

Abraham Nyska
ATTN: Sherry Thaxton
Luxembourg - Pamol, Inc.
5100 Poplar Avenue - Suite 2746
Memphis, TN 38137
901-761-9475
Fax: 901-761-9477

Joanne Otani
Senior Associate Health Scientist
ChemRisk
8500 Brooktree Road - Suite 300
Wexford, PA 15090-9287
412-934-3744
Fax: 412-934-5944
E-mail: joanne_otani@
mclaren-hart.com

Elizabeth Owens
ISK Biosciences Corporation
5966 Heisley Road
Mentor, OH 44061-8000
216-357-4188
Fax: 216-357-4692
E-mail: owense@iskbc.com

George Parris
American Wood Preservers Institute
2750 Prosperity Avenue - Suite 550
Fairfax, VA 22031-4312
703-204-0500
Fax: 703-204-4610

Tim Powers
Roth Associates
6115 Executive Boulevard
Rockville, MD 20852
301-770-4405
Fax: 301-770-9248

Jackie Randall
Environmental Specialist
Arnold & Porter Law Firm
555 Twelfth Street
Washington, DC 20004
202-942-5933
Fax: 202-942-5999

Randy Roth
Manager
Corporate Health and Safety Division
ARCO
444 South Flower Street
Los Angles, CA 90071
213-486-8733
Fax: 213-486-6402
E-mail: rrothl@is.arco.com

Ed Ruckert
McDermott, Will & Emery
1850 K Street, NW - Suite 500
Washington, DC 20006
202-778-8214
Fax: 202-778-8017

William Ruoff
Project Risk Assessor
Woodward Clyde International
Stanford Place 3
4582 South Ulster Street
Denver, CO 80237
303-740-2786
Fax: 303-694-3946
E-mail: wlruoff0@wcc.com

Anne Shimabukuro
Project Assistant
Public Health Program
The Natural Resources
Defense Council
1200 New York Avenue, NW
Suite 400
Washington, DC 20005
202-289-2387
Fax: 202-289-0990
E-mail: ashimabukuro@nrdc.org

Robert Sielaty
Compliance Services International
2001 Jefferson Davis Highway
Suite 1010
Arlington, VA 22202-3603
703-415-4600
Fax: 703-415-1767
E-mail: rsielaty@
complianceservices.com

Tracey Slayton
Toxicologist
Gradient Corporation
216 Bloomfield Street
Hoboken, NJ 07030
201-653-6885
Fax: 201-653-7335
E-mail: tslayton@cam.gradcorp.com

Bonnie Stern
Senior Scientist/Manager
Risk Sciences and Management
EA Engineering, Science,
and Technology
Metro One Plaza
8401 Colesville Road - Suite 500
Silver Spring, MD 20910
301-565-4216
Fax: 301-587-4752

Jim Taft
Chief
Office of Ground Water
and Drinking Water
U.S. Environmental Protection Agency
401 M Street, SW (4607)
Washington, DC 20460
202-260-5519
Fax: 202-260-3762
E-mail: taft.jim@epamail.epa.gov

Shirley Tao
Toxicologist
Division of Programs and
Enforcement Policy
Contaminant Standards
Monintoring and Programs
Food and Drug Administration
200C Street, SW (HFS-308)
Washington, DC 20204
202-205-2972
Fax: 202-260-0498

Marcia van Gemert
Director, Toxicology
Charles, Conn, & van Gemert, LLC
12521 Old Homeplace Drive
Charlotte Hall, MD 20622
301-934-4990
Fax: 301-934-5687
E-mail: mvang@ix.netcom.com

Bernard Wagner
President
Wagner Associates, Inc.
343 Millburn Avenue
Courtyard - Suite 208
Millburn, NJ 07041
201-564-5258
Fax: 201-564-5292

Pat Ware
Editor
Bureau of National Affairs
1231 25th Street, NW
Room 338-S
Washington, DC 20037
202-452-4401
Fax: 202-452-5331

Jim White
Toxicologist
Washington State
Department of Health
P.O. Box 47846
Olympia, WA 98504-7846
360-753-2396
Fax: 360-586-4499
E-mail: jw0303@hub.doh.wa.gov

Paul White
Environmental Scientist
National Center for
Environmental Assessment
U.S. Environmental Protection Agency
401 M Street, SW (8623)
Washington, DC 20460
202-260-2589
Fax: 202-260-3803

Jeannette Wiltse
Director, Health and
Ecological Criteria Division
Office of Water
U.S. Environmental Protection Agency
401 M Street, SW (4304)
Washington, DC 20460
202-260-7317
Fax: 202-260-1036

Jennifer Wu
Standards and Risk
Management Division
Office of Ground Water
and Drinking Water
U.S. Environmental Protection Agency
401 M Street, SW (4607)
Washington, DC 20460
202-260-0452
E-mail: wu.jennifer@epamail.epa.gov

Appendix B

LIST OF ARTICLES REVIEWED BY EXPERT PANELISTS

List of Articles Used as References

Note: Bold articles are in press

1 **Rossman, T. G., E. I. Goncharova, T. Rajah, and Z. Wang. 1997. Human cells lack the inducible tolerance to arsenite seen in hamster cells. Mutation Research. In press.**

2 Rossman, T. G. 1994. Metal mutagenesis. In *Toxicology of Metals—Biochemical Aspects,* R.A. Goyer and M.G. Cherian, eds. New York, NY: Springer-Verlag. pp. 374-405.

3 Rossman, T. G., and D. Wolosin. 1992. Differential susceptibility to carcinogen-induced amplification of SV40 and *dhfr* sequences in SV40-transformed human keratinocytes. Molecular Carcinogenesis 6:203-13.

4 **Rossman, T. G. 1997. Molecular and genetic toxicology of arsenic. In *Environmental Toxicology*, J. Rose, ed. Gordon and Breach, Publishers. In press.**

5 **Crecelius, E., and J. Yager. 1997. Intercomparison on analytical methods for arsenic speciation in human urine. Environmental Health Perspective. In Press.**

6 **Gonsebatt, M. E., L. Vega, A. M. Salazar, R. Montero, P. Guzman, J. Blas, L. M. Del Razo, G. Garcia-Vargas, A. Albores, M. E. Cebrian, M. Kelsh, and P. Ostrosky-Wegman. 1997. Cytogenetic effects in human exposure to arsenic. Mutation Research. In Press.**

7 Huang, R., I. Ho, L. Yih, and T. Lee. 1995. Sodium arsenite induces chromosome endoreduplication and inhibits protein phosphatase activity in human fibroblasts. Environmental and Molecular Mutagenesis 25:188-96.

8 Implications of Arsenic Genotoxicity for Dose Response of Carcinogenic Effects

9 Dong, J., and X. Luo. 1994. Effects of arsenic on DNA damage and repair in human fetal lung fibroblasts. Mutation Research, DNA Repair 315:11-15.

10 Kochhar, T.S., W. Howard, S. Hoffman, and L. Brammer-Carleton. 1996. Effect of trivalent and pentavalent arsenic in causing chromosome alterations in cultured Chinese hamster ovary (CHO) cells. Toxicology Letters 84:37-42.

11 Repetto, G., P. Sanz, and M. Repetto. 1994. Comparative in vitro effects of sodium arsenite and sodium arsenate on neuroblastoma cells. Toxicology 92:143-53.

12 Oya-Ohta, Y., T. Kaise, and T. Ochi. 1996. Induction of chromosomal aberrations in cultured human fibroblasts by inorganic and organic arsenic compounds and the different roles of glutathione in such induction. Mutation Research 357:123-29.

13 Lerda, D. 1994. Sister-chromatid exchange (SCE) among individuals chronically exposed to arsenic in drinking water. Mutation Research 312:111-20.

14 Vega, L., M. E. Gonsebatt, and P. Ostrosky-Wegman. 1995. Aneugenic effect of sodium arsenite on human lymphocytes in vitro: An individual susceptibility effect detected. Mutation Research 334:365-73.

15 Wang, T. S., and H. Huang. 1994. Active oxygen species are involved in the induction of micronuclei by arsenite in XRS-5 cells. Mutagenesis 9:253-57.

16 Meng, Ziqiang, Hsie, Abraham. 1996. Polymerase chain reaction-based deletion analysis of spontaneous and arsenite-enhanced gpt mutants in CHO-AS_{52} cells. Mutation Research 356: 255-259.

17 Wang, Zaolin, Dey, Saibal, Rosen, Barry, and T. Rossman. 1996. Efflux-mediated resistance to arsenicals in arsenic-resistant and -hypersensitive chinese hamster cells. Toxicology and Applied Pharmacology 137: 112-119.

18 **Healy, S. M., R. A. Zakharyan, H. V. Aposhian, Enzymatic methylation of arsenic compounds: IV. in vitro and in vivo deficiency of the methylation of arsenite and monomethylarsonic acid in the guinea pig. Mutation Research. In Press.**

19 **Aposhian, H. V. 1997. Enzymatic methylation of arsenic species and other new approaches to arsenic toxicity. Annual Review Pharmacology, Toxicology. In Press.**

20 Landolph, J. R. 1993. Molecular mechanisms of transformation of C3H/10T1/2 C1 8 mouse embryo cells and diploid human fibroblasts by carcinogenic metal compounds. Environmental Health Perspectives. 119-125.

21 Hartmann, A., and G. Speit. 1996. Effect of arsenic and cadmium on the persistence of mutagen-induced DNA lesions in human cells. Environmental and Molecular Mutagenesis 27: 98-104.

22 Cavigelli, M., W.W. Li, A. Lin, B. Su, K.Yoshioka, and M. Karin. 1996. The tumor promoter arsenite stimulated AP-1 activity by inhibiting a JNK phosphatase," The EMBO Journal 15:22 6269-6279.

23 Brown, J., K. Kitchin. 1996. Arsenite, but not cadmium, induces omithine decarboxylase and heme oxygenase activity in rat liver: relevance to arsenic carcinogenesis. Cancer Letters 227-231.

24 Styblo, Miroslav, Serves, Spiros, Cullen, William, Thomas, J. David. 1997. Comparative inhibitions of yeast glutathione reductase by arsenicals and arsenothiols. Chem. Res. Toxicol 27-33.

25 Mann, Sabine; Droz, Pierre-Olivier; and M. Vahter. 1996. A pysiologically based pharmacokinetic model for arsenic exposure II. validation and application in humans. Toxicology and Applied Pharmacology 471-486.

26 Tice, Raymond, R., J.W. Yager, P. Andrews, E. Crecelius. 1997. Effect of hepatic methyl donor status on urinary excretion and DNA damage in B6C3F1 mice treated with sodium arsenite. Mutation Research. In Press.

27 Hughes, Michael, D. Thompson. 1996. Subchronic dispositional and toxicological effects of arsenate administered in drinking water to mice. Journal of Toxicology and Environmental Health 49: 177-196.

28 Wiencke, John K., J.W. Yager, A. Varkonyi, M. Hultner, L. Lutze. 1997. Study of arsenic mutagenesis using the plasmid shuttle vector pZ189 Propagated in DNA Repair Proficient Human Cells. Mutation Research. In Press.

29 Yager, Janice W, and J.K. Wiencke. 1993. Enhancement of chromosomal damage by arsenic: Implications for mechanism," Environmental Health Perspectives Supplements 101: 79-82.

30 Yager, Janice W., J.K. Wiencke. 1997. Inhibition of poly (ADP-RIBOSE) polymerase by arsenite. Mutation Research. In Press.

31 S. Mann, P.O. Droz, and M. Vahter. 1996. A pysiologically based pharmacokinetic model for arsenic exposure I. development in hamsters and rabbits. Toxicology and Applied Pharmacology 137: 8-22.

32 Mass, Marc J., and L. Wang. Arsenic alters cytosine methylation patterns of the promoter of the tumor suppressor gene p53 in human lung cells: A model for mechanism of carcinogenesis. Mutation Research. In Press.

33 Ramirez, P., D.A. Eastmond, J.P. Laclette, and P. Ostrosky-Wegman. Disruption of microtubule assembly and spindle formation as a mechanism for the induction of aneuploid cells by sodium arsenite and vanadium pentoxide. Mutation Research. In Press.

34 Biggs, Mary Lou, D.A. Kalman, L.E. Moore, C. Hopenhayn-Rich, M.T. Smith, A.H. Smith. Relationship of urinary arsenic to intake estimates and a biomarker of effect, bladder cell micronuclei. Mutation Research. In Press.

35 Rasmussen, Ronald E., D.B. Menzel. Variation in arsenic-induced sister chromatid exchange in human lymphocytes and lymphoblastoid cell lines. Mutation Research. In Press.

36 Germolec, Dori R., J. Spalding, G.A. Boorman, J.L. Wilmer, T. Yoshida, P.P. Simeonova, A. Bruccoleri, F. Kayama, K. Gaido, R. Tennant, F. Burleson, W. Dong, R.W. Lange, and M.I. Luster. Arsenic can mediate skin neoplasia by chronic stimulation of keratinocyte-derived growth factors. Mutation Research. In Press.

37 Moore, Martha M., K. Harrington-Brock, and C.L. Doerr. Relative genotoxic potency of arsenic and its methylated metabolites. Mutation Research. In Press.

38 Chiou, Hung-Yi, LL. Hsieh, LI. Hsu, YH. Hsu, FI. Hsieh, ML. Wei, HC. Chen, Y. Chih, Hui-Ting; LC. Leu, YM. Hsueh, C.C. Wu, MH. Tang, and CJ. Chen. Arsenic methylation capacity, body retention, and null genotypes of glutathione S-transferase M1 and T1 among current arsenic-exposed residents in Taiwain. Mutation Research. In Press.

39 **Hsu, Yi-Hsiang; SY. Li, HY. Chiou, PM. Yeh, YM. Hsueh, CJ. Chen, Spontaneous and induced sister chromatid exchanges and delayed cell proliferation in peripheral lymphocytes of skin cancer patients and matched controls of arseniasis-hyperendemic villages in Taiwan. Mutation Research. In Press.**

40 EPA's *Federal Register* Tuesday, April 23, 1996.

41 Memorandum on EPA Research Plan for Arsenic in Drinking Water to the Board of Scientific Counselors Ad-Hoc Committee on Arsenic Research, from William Farland, NCEA, January 2, 1997.

42 EPA Draft Drinking Water Criteria.

43 **Mass, Mark, J., and L. Wang. 1997. Arsenic alters cytosine methylation patterns of the promoter of the tumor suppressor gene in human lung cells: A model for mechanism of carcinogenesis. Mutation Research. In Press.**

44 Arsenic, inorganic; CASRN 7740-38-2 article

45 1994. Metallothionein - II and Ferritin H mRNA levels article, Biochemical and Biophysical Research Communications 205:1.

46 Hsueh, Y-M, G-S. Cheng, M-M. Wu, H-S.Yu, T-L. Kuo, and C-J. Chen. 1995. Multiple risk factors associated with arsenic-induced skin cancer: Effects of chronic liver disease and malnutritional status. British Journal of Cancer 71: 109-114.

47 Albores, A., C. Sinal, G. Cherian, and J.R. Bend. 1995. Selective increase of rat lung cytochrome P450 1A1 dependent monooxygenase activity after acute sodium arsenite administration. Can. J. Physiol. Pharmacol 73.

48 Yamamoto, S., Y. Konishi, T. Matsuda, T. Murai, M-A. Shibata, I. Matsui-Yuasa, S. Otani, K. Kuroda, G. Endo, and S. Fukushima. 1995. Cancer induction by an organic arsenic compound, dimethylarsinic acid (cacodylic acid), in F344/DuCrj rats after pretreatment with five carcinogens. Cancer Research 55: 1271-1276.

49 Devereaux, T., C.M. White, R.C. Sills, J.R. Bucher, R.R. Maronpot, M.W. Anderson. 1994. Low frequency of H-ras mutations in hepatocellular adenomas and carcinomas and in hepatoblastomas from B6C3F1 mice exposed to oxazepam in the diet. Carcinogenesis 15:5 1083-1087.

50 Hartmann, A., and G. Speit. 1996. Effect of arsenic and cadmium on the persistence of mutagen-induced DNA lesions in human cells. Environmental and Molecular Mutagenesis 27:98-104.

51 Van Wijk, R., M. Welters, J.E.M. Souren, H. Ovelgonne, and F.A.C. Wiegant. 1993. Serum-stimulated cell cycle progression and stress protein synthesis in C3H10T1/2 fibroblasts treated with sodium arsenite. Journal of Cellular Physiology 155: 265-272.

52 Dulout, F.N., C.A. Grillo, A.I. Seoane, C.R. Maderna, R. Nilsson, M. Vehter, F. Darroudi, A. Natarajan, T. Adayapalam. 1996. Chromosomal aberrations in peripheral blood lymphocytes from native andean women and children from northwestern argentina exposed to arsenic in drinking water. Mutation Research 370: 151-158.

53 Vega, L., M.E. Gonsebatt, P. Ostrosky-Wegman. 1995. Aneugenic effect of sodium arsenite on human lymphocytes in vitro: An individual susceptibility effect detected. Mutation Research 334: 365-373.

54 Gurr, J-R., Y-C., Lin, I-C. Ho, K-Y. Jan, and T-C. Lee. 1993. Induction of chromatid breaks and tetraploidy in chinese hamster ovary cells by treatment with sodium arsenite during the G2 phase. 1993. Mutation Research 319: 135-142.

55 Lundberg, K.S., P.L. Kretz, S. Provost, and J.M. Short. 1993. The use of selection in recovery of transgenic targets for mutation analysis. Mutation Research 301: 99-105.

56 Cobo, J.M.; J.G. Valdez, L.R. Gurley. 1995. Inhibition of mitotic-specific histone physphorylation by sodium arsenite. Toxicology In Vitro 9:4 459-465.

Appendix C

PRE-MEETING COMMENTS

H. Vasken Aposhian

Arsenic Carcinogenicity Comments

There are many problems concerning arsenic and cancer of which we are virtually ignorant and which need extensive study.

1. Arsenic speciation. Which species, arsenate, arsenite, methylarsonic acid containing As^V (MMA^V) or dimethylarsinic acid containing As^V (DMA^V) cause cancer or are promoters? These are the most recognized of the arsenic species. However, MMA^{III} (and even DMA^{III}) is certainly an intermediate in arsenite methylation but it has neither been measured or isolated in vivo. It has been chemically synthesized and is available but no studies on its mutagenic and carcinogenic properties have been performed. Studies using 400 ppm DMA^V and claiming cancer induction in rats after pretreatment with five carcinogens can be criticized not only because of the very high DMA dose but also because the rat is not considered to be a good model system for studying arsenic as stated below.

2- Animal species studied. Many investigators have studied the mutagenic and carcinogenic effects of arsenic in the rat. These results should be viewed with a great deal of caution, if not outright irrelevance as a model system for the human. The rat is unique in that DMA is bound and acccumulates in the red cells. The National Research Council (1) has recommended that the rat not be used to study arsenic metabolism because of the rat's unique handling of DMA.

3- The diversity of the methylating enzymes of arsenate/arsenite metabolism. With the recent purification and characterization of arsenite methyltransferases (2,3), meaningful experiments could be performed. These enzymes, which are found in the liver of rabbit, rat, mouse, hamster, pigeon and rhesus monkey, are deficient in the marmoset monkey, tamarin monkey, squirrel monkey, chimpanzee, and guinea pig (3,4, 5). The literature often states that methylation only takes place in the liver. It has now been demonstrated that in the male mouse, the specific activity of these enzymes is greatest in the testis>kidney>liver=lung (6). What is disturbing is that such activity has not been found in human liver as yet. Whether this is due to the unavailability of fresh human liver (removed within 1 hr of death) or other reasons is unknown at the present time. Because of the extensive species diversity as to the amount of arsenic species excreted in the urine, and the diversity as to the amount of the arsenite methyltransferases in the livers of a number of animal species, the use of human tissues and other human studies should be encouraged by increasing the resources to elucidate some of the questions dealing with the metabolism, mutagenicity and carcinogenicity of arsenic species. The availability of freshly harvested human tissue is, to say the least, very limited but urgently needed. Not only is the tissue expensive when available but the logistics of tracking it down is extremely time consuming.

LITERATURE:

1-National Research Council Report. Medical and Biologic Effects of Environmental Pollutants, Arsenic National Academy of Sciences: Washington, DC, 1977

2-ZAKHARYAN, R. A., WU, Y., BOGDAN, G. M. AND APOSHIAN, H. V.: Enzymatic methylation of arsenic compounds. I: Assay, partial purification, and properties of arsenite methyltransferase and monomethylarsonic acid methyltransferase of rabbit liver. Chem. Res. Toxicol. 8: 1029-1038, 1995.

3-ZAKHARYAN, R. A., WILDFANG, E. AND APOSHIAN, H. V.: Enzymatic methylation of arsenic compounds: III. The marmoset and tamarin, but not the rhesus, monkey are deficient in methyltransferases that methylate inorganic arsenic. Toxicol. Appl. Pharmacol. 140: 77-84, 1996.

4-HEALY, S. M., ZAKHARYAN, R. A. AND APOSHIAN, H. V.: Enzymatic methylation of arsenic compounds: IV. In vitro and in vivo deficiency of the methylation of arsenite and monomethylarsonic acid in the guinea pig. Mutation Res. In press, 1997.

5-APOSHIAN, H. V.: Enzymatic methylation of arsenic species and other new approaches to arsenic toxicity. Annu. Rev. Pharmacol. Toxicol. 37: 397-419, 1997.

6- HEALY, S. M., CASAREZ, E. A., AYALA-FIERRO AND APOSHIAN, H. V.: Arsenite methyltransferase activity in tissues of mice. SUBMITTED

Samuel Cohen

Samuel M. Cohen, M.D., Ph.D.

COMMENTS REGARDING CANCER RISK ASSESSMENT OF ARSENIC.

There are several aspects of the cancer risk assessment for arsenicals in humans which will be better covered by experts in those respective areas. I will provide a brief summary of some of these points as they pertain to my interpretation, but I will focus primarily on the studies involving carcinogenicity of arsenicals.

Arsenic has been identified by epidemiologic and case reports as being associated with the development of cancer in humans, most notably the development of skin cancer, but more recently with the possible development of cancer of internal organs, especially the urinary bladder, but also liver, kidney, and possibly colon. Extrapolating from experimental results and epidemiologic studies to estimates of possible cancer risks to humans exposed to low doses of arsenic is the critical issue. The reports of cancer in humans related to arsenic exposure have been at high doses, whereas all people are exposed to arsenic at some level, usually at much lower than the exposure levels reported associated with cancer development.

There is considerable evidence that inorganic arsenicals are more toxic than the organic forms. Metabolically, there is rapid interconversion between Arsenic 5 and Arsenic 3, as well as mono and dimethylation of the arsenic species *in vivo*. Variations in metabolism between species have been identified.

A critical factor in evaluating the carcinogenicity of arsenicals is determination of genotoxicity. For the most part, arsenic and arsenicals are considered to be non-genotoxic, i.e., they do not react directly with DNA. *In vitro* and *in vivo* short term screens for genotoxicity have been negative. However, indirect damage to chromosomes has been reported, including positive results in studies for chromosomal aberrations, micronuclei, and sisterchromatid exchange. Because of the indirect nature of the damage to the chromosomes, there has been a general consensus that the effects of arsenicals is a high dose (relatively speaking) phenomenon. The issue of dose extrapolation is a key to the decisions regarding establishment of "safe" levels of human exposure in the drinking water.

Epidemiologic studies have clearly shown a relationship between arsenic exposure and the development of cancer in humans. However, there are numerous difficulties in trying to draw conclusions with respect to the dose response. For the most part, a careful identification of the exposure levels of the experimental and control groups has not been accomplished, partly due to difficulty in measuring arsenic levels and metabolites, but also in trying to establish biomarkers indicative of long term exposure. In addition, several of the epidemiologic studies involving cancers of internal organs have failed to take into account confounding factors. For example, in studies on bladder cancer, identification of the cigarette smoking status of the individuals is essential, as is exposure to other potential environmental chemicals related to the development of bladder cancer. With liver cancer, it is essential that there be an identification of other potential toxicities to the liver, such as aflatoxin and nitrosamine exposures, but more importantly, the association with hepatitis B and C viruses and with heavy alcohol consumption. Careful delineation of these confounding factors in any study is essential.

Until recently, considerable difficulty has been involved with risk assessment of arsenic because of the lack of an appropriate animal model. It is only in the past few years that there has been association of an increased risk of certain types of cancer in animals exposed to various doses of arsenicals. In general, this has usually involved administration of dimethylarsenic acid (DMA) to rats or mice. In the studies by Fukushima et al., this has been most commonly related to development of bladder cancer, kidney cancer, or liver cancer following administration of DMA after prior administration with a known carcinogen, such as N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN). In these studies, it has generally been observed that doses above 10 PPM in the drinking water have been associated with an increased risk of cancer development in various tissues, but doses below 10 PPM have generally been negative. The results at dose exposure of 10 PPM have been equivocal. Importantly, in his and other published studies, DMA has not been carcinogenic, although a full two year bioassay has not been published for these studies. Yamanaka et al., have shown that DMA administered after 4-nitroquinoline 1-oxide increased the risk of lung tumors in mice, with a dose response suggesting that doses above 10 PPM are positive but those below are negative. DMA by itself was negative.

All of these results in animals taken together suggest that the carcinogenicity of arsenicals appears only at relatively high doses. This in keeping with its apparent non-genotoxicity and the relationship of the carcinogenicity to increased cell proliferation, possibly secondary to regenerative hyperplasia following toxicity. These animal models should be able to provide a resource for more definitive studies on the mechanisms of action involved in the carcinogenicity of arsenicals.

In animal studies, the issue has yet to be resolved as to why an organic arsenical, DMA, has led to an increased risk of certain types of cancer in contrast to the lack of effect with inorganic arsenicals. Recently, results from the laboratory of Dr. Shoji Fukushima in Osaka City University Medical School, in Japan, suggest that it may be related to the metabolism of these different agents in the rat. DMA leads to a so far unidentified metabolic product at high concentrations in the urine which is not present in animals exposed to inorganic arsenic. The doses of inorganic arsenic that are administered are relatively low compared to those of DMA because of acute toxicity related to the inorganic arsenical. Careful studies regarding the metabolism and toxicity of the different arsenicals should be helpful in resolving issues regarding relative risks in the different arsenicals.

The results in animal studies and the genotoxicity support the hypothesis that in humans, arsenicals pose a carcinogenic hazard only at relatively high doses. The difficulty, is that we currently do not have the data necessary for identifying what an appropriate "low" dose is for human exposures. This will require much greater detail regarding exposure levels in highly exposed populations, as well as careful epidemiologic studies taking into account confounding factors.

All of the data so far strongly support a mode of action suggestive of a nonlinear dose response. This is supported by the studies based on direct genotoxicity, animal experimentation, and human epidemiology. The possibility that arsenic is an essential dietary component also supports the hypothesis that low dose exposures do not pose a carcinogenic hazard to humans.

In summary, there is strong evidence that arsenic is a human carcinogen, but the evidence supports the concept that it is at a relatively high dose compared to low dose exposure. There is considerable uncertainty, however, as to what the dose response relationship is from the relatively high doses to the usual levels of exposure in human populations due to drinking water consumption.

Jean-Pierre Issa

Introduction

Arsenic (As) is classified as a human carcinogen based on epidemiologic studies of populations exposed to relatively high levels of As from contaminated water supply, or through inhalation. Arsenic appears to act as a carcinogen primarily through non-mutagenic pathways, but the precise mechanism of action (MOA) of As in causing tumors remains unclear. The primary charge to the reviewers on this panel is to review the available evidence for a mechanism of action relating As to carcinogenicity. Secondary goals include a determination of the proper dose-response relationship that exists for this MOA (liner vs. non-linear). I will limit my comments here to my general areas of expertise.

General Comments

Determining the relevant MOA of any carcinogen is a difficult task because of the limitations of in-vitro analysis, and the difficulties in performing/interpreting in-vivo studies. This is particularly difficult for a non-mutagenic carcinogen, and is quite challenging for As given the lack of an appropriate animal model for cancer induction by this metal compound. Nevertheless, a large body of data has now linked As exposure with various physiologic alterations that each could be responsible for the enhanced cancer rate in exposed individuals. However, the most compelling data linking a particular carcinogen exposure with in-vivo tumorigenesis comes from detailed studies of molecular alterations and mutational spectra in tumors from patients exposed to the carcinogen under study. Such studies have revealed 'signature' lesions for some carcinogens such as Aflatoxin and UV radiation, and have confirmed the important role these agents play in human tumors. Unfortunately, very few studies have addressed this issue for As related tumors. In the absence of this crucial data, any proposed MOA for As related carcinogenicity must be considered tentative.

Specific Comments

In this section, I will address some of the issues related to determining a MOA for AS related carcinogenicity, and discuss in some detail the proposed link between As exposure and aberrant DNA methylation.

1. <u>Is As a mutagen?</u> Most studies performed over the past two decades have found no appreciable mutagenicity for arsenite or arsenate. While the bulk of the evidence suggests that

As is non-mutagenic, a recent study (Wiencke et al.) using a novel test for mutagenesis revealed that, at 5 μm concentration, arsenite induced a significant increase in the frequency of mutations. While this could potentially be due to altered DNA repair or oxidative damage to the cell (see below), further studies of arsenite effects on mutagenesis using novel sensitive assays appear to be warranted. Nevertheless, the present consensus that arsenic is essentially non-mutagenic appears to be solid.

2. As and DNA repair. There is ample evidence from multiple studies suggesting that As delays and/or inhibits the repair of DNA damage induced by various agents, and increases mutation rates synergistically with several common carcinogens. The interaction between As and UV radiation is especially interesting since skin tumors are one of the most common features of As carcinogenicity. The mechanism of this As induced inhibition of DNA repair may well be due to its inhibition of DNA ligase and/or other enzymes involved in DNA synthesis and repair. Alternatively, As may potentiate oxidative damage to DNA through an unknown mechanism. While this effect of As on DNA repair may be considered a leading possibility for a MOA of As carcinogenesis, until the mutational spectrum of several genes in As related tumors is known, this conclusion must be considered unproven. In particular, some studies have suggested that As delays the repair of UV induced pyrimidine dimers. If that is the case, then such mutations should be prominent in the spectrum of P53 gene mutations in the skin tumors of exposed individuals. What little data exists to address this has not confirmed this hypothesis (see below).
3. As and DNA methylation. Cytosine DNA methylation within promoters has recently emerged as an alternative mechanism for inactivating tumor-suppressor genes. Nickel, another non-genotoxic carcinogen appears to induce aberrant DNA methylation and chromatin changes that have been postulated to play a role in its carcinogenicity. One study (Mass et al.) has addressed the effects of As on DNA methylation, and the DNA methylation machinery. In this study, it was found that As induced (1) increased Cytosine-5-DNA-Methyltransferase activity, (2) increased overall cytosine methylation and (3) apparent de-novo methylation in the promoter of the P53 gene. Strikingly, this de-novo methylation involved cytosines that were not part of the CpG dinucleotide, an extremely uncommon occurrence in human tissues. The authors speculated that As carcinogenicity may relate in part to its effects on DNA methylation. These data, however have to be interpreted with some caution; Increased Cytosine-Mtase activity and changes in overall DNA methylation are relatively non-specific

events that have been seen with a variety of carcinogens, and can be observed if a cell is induced to proliferate. P53 promoter methylation is a very intriguing finding. There are, however some technical limitations inherent to the assays utilized, and the data needs to be confirmed using other methods, and perhaps other genes. Most importantly, however, P53 promoter methylation has never been reported in human cancers. This proposed mechanism of action must therefore be considered speculative until a thorough analysis of human tumors from patients exposed to As is performed. In particular, if P53 mutations are rare in these tumors, and P53 methylation is found in its promoter, than this molecular lesion could be considered a signature lesion for As exposure. Again, the little data available on P53 mutations in As related tumors is conflicting in this regard.

4. <u>Other potential MOA for As carcinogenesis.</u> As induces increased chromosomal aberrations in exposed cells, including large chromosomal changes, sister chromatid exchanges, micronuclei formation and gene amplification. Some of these changes can be detected in exfoliated cells in urine specimens from exposed patients. These anomalies are relatively non-specific and not always reproducible. They may also partly relate to As effects on DNA repair. As also appears to potentiate oxidative DNA damage, and this again could contribute to its carcinogenicity. As has also been shown to induce changes in gene expression, including upregulation of heat-shock proteins and some growth factors. Whether these changes are a simple response to As toxicity, and whether they could contribute to carcinogenesis is unknown. Nevertheless, it is possible that As increases the incidence of cancer by a mechanism totally unrelated to its effects on DNA. For example, it is possible that As toxicity causes tissue remodeling with chronic stimulation of otherwise quiescent stem cells. This inflammation/injury type of response could lead to an increased cancer rate, independent of DNA effects.
5. <u>Mutational spectra in As related tumors.</u> Only two small studies have addressed this issue. Shibata et al. have sequenced the p53 gene in 13 urothelial tumors in an area endemic for black foot disease that is related to high As in well water. 8 cases had mutations, with a similar spectrum as urothelial tumors from unexposed patients. No correlation with As exposure was reported in this study. Hsieh et al. studied Ras and p53 mutations in 16 As-related skin tumors. No mutations were found, which is distinctly different from UV related skin tumors and, if confirmed in other larger studies, weakens altered DNA repair as a MOA for As carcinogenesis. Thus, these two small studies reached opposite conclusions. To my

knowledge, no studies on chromosomal aberrations, loss of heterozygosity, microsatellite instability or promoter methylation have been reported on As related tumors.

Conclusions

While the epidemiologic data linking As and carcinogenesis is strong, a specific MOA for this association remains somewhat speculative at the present time. DNA repair alterations may be an attractive candidate for this MOA, but a more definitive answer will have to await careful molecular studies in tumors from patients exposed to As. It is recommended that a bank of tumors from patients exposed to high levels of As and control patients be established to facilitate such molecular studies (p53/Ras/Patched mutations, P16 deletion/methylation/mutation, gross gene amplification/deletion by Comparative Genomic Hybridization etc.).

Andres Klein-Szanto

Preliminary Report on Arsenic Carcinogenicity May 1, 1997

A. Klein-Szanto

Pre-meeting Comments

The charge to the panel contains a number of issues that directly or indirectly pertain to the modes of action (MoA) of arsenic carcinogenicity. Thus, my preliminary comments are generic and aimed at summarizing my reaction to the proposed MoA's found in the recent literature.

Mechanism of Action:

A preliminary glance at the literature indicates that relatively little is known and that the information is fragmented and inconclusive. There are many papers demonstrating a variety of pleiotropic effects in vitro, all or most of which are compatible with properties of tumor promoters. In addition there are a couple of in vivo studies suggesting the same mechanism. Since the mechanisms of tumor promotion are rather complex, this does not necessarily clarify the issue.

A review of the papers published in the last ten years show a preference for the following three modes of action (MoA):

1)Induction of active oxygen species.
2)Alterations induced by methylation related to biotransformation of arsenical compounds.
3) Effects on DNA repair mechanisms

Induction of active oxygen species.

The induction of free radicals by arsenic exposure is quite well documented in experimental work.
The role of active oxygen species has been researched in the past and has many adherents who have proposed a significant role of free radicals in multi-stage carcinogenesis and specifically during tumor promotion. Although free radicals have tumor promoting effects in the traditional skin carcinogenesis model, they are not very strong promoters. Active oxygen species also induce ornithine decarboxylase (ODC), which is widely accepted as a marker of cell proliferation and tumor promoting effects. DMA and arsenite have been shown to increase ODC activity in rat tissues, thus directly or indirectly through free radical formation, there is evidence that arsenic compounds could be regarded as tumor promoters. The work of Fukushima's laboratory certainly shows the tumor enhancing or promoting effect of DMA in vivo.
Free radicals are also genotoxic and a combination of the promoting plus genotoxic effects could indeed account for the carcinogenicity of arsenic.

Alterations induced by methylation

Detoxification of arsenic occurs through methylation and the methylated compound DMA has been shown to be a tumor promoter-like agent in rat urinary bladder, liver, etc. It has been speculated that the methylation pathway of biotransformation of arsenic could in some way interfere with methylation of DNA, eventually leading to hypermethylation or hypomethylation of important target genes. The only paper providing preliminary evidence for this MoA is the one from Dr. Mass indicating that exposure of a human lung adenocarcinoma cell line to inorganic arsenic produces hypermethylation of the promotor region of the p53 gene.
Although this finding is exciting, much more work is needed to clarify this putative MoA.

Effects on DNA repair mechanisms

Although arsenic compounds are usually described as being non-mutagenic in the mammmalian and bacterial test systems, there is an increasing body of literature indicating increased mutagenicity and clastogenicity when arsenic is used with other mutagens. Dr. Rossman has worked in this field and is much better qualified to review this area.
Similarly, there are reports describing the inhibitory effects of arsenic on DNA repair processes.
This MoA does not seem to be very popular in some reviews on this topic. Nevertheless, it should be considered as a an important and probable MoA given the numerous reports on chromosomal abnormalities in human populations exposed to arsenic in the drinking water.

Tentative Conclusion

An optimistic view is that any of these MoA's alone or combined could account for the carcninogenicity of arsenic. On the other hand a pessimist could justly assert that most of the evidence for these MoA's is insufficient and even sometimes anecdotal. Most of the evidence although inconclusive, point to arsenic as a tumor promoter. Fukushima and collaborators (Yamamoto et al. and Wanibuchi et al.), in the only successful study of its kind, have certainly showed a tumor promotion- like effect of DMA in rats.
My conclusion is that more work is needed and that these three MoA's are likely but not necessarily the final candidates that will explain arsenic carcinogenicity.

Julian Preston

No 22 13 #14

Expert Panel on Arsenic Carcinogenicity: Premeeting Comments

R. Julian Preston, CIIT

Arsenic is unusual in that it is described as being a human carcinogen but not a rodent carcinogen when tested in a standard two-year bioassay. Thus, the classification as a human carcinogen in this case relies exclusively on epidemiological data, for which a fairly extensive literature is clearly indicative of a role for arsenic in tumor induction. These studies are essentially the hazard identification step for a risk assessment, with less reliability for dose response determination, especially at low environmental exposures. To begin to describe the dose response curve for arsenic-induced tumors it is necessary to use surrogates for the tumor response. It can be argued that since cancer is a genetic disease, the result of a series of mutations (point mutations, structural and numerical chromosome alterations) then data for arsenic-induced mutations should provide a qualitative estimate of dose response curve shape for tumor induction at low exposures.

In addition to selecting the appropriate response endpoint for use in dose response assessment, it is also necessary to establish the appropriate measure for effective dose. The human studies have been rather inconclusive as regards arsenic exposure, and so here ingested or inhaled dose is largely unknown. Arsenic is converted to methylated forms that are excreted, and it appears that this is more effective in rodents than humans, providing a possible explanation for species differences in response, given that the methylated forms appear to be less genotoxic than inorganic arsenic. Thus, effective dose might be the amount of inorganic arsenic in the cells of a target tissue, or at least, for in vivo genotoxicity studies, amount of inorganic arsenic in the tissue being analyzed.

A range of genotoxicity studies point to arsenic being genotoxic and also acting as a comutagen, quite possibly through inhibition of DNA repair processes or at the level of transcription. As an *initial* consideration of the risk assessment for arsenic at low exposures, it would seem to be more appropriate to consider its genotoxicity, and then to consider its comutagenic effects as a secondary assessment.

In an ideal sense, a complete understanding of the mechanism by which arsenic induces genetic alterations (and cancer) is needed to define the cancer risk at all exposure levels. However, it is to be noted that, in the absence of such a complete understanding, knowledge of the mode of action can be used to reduce the uncertainty in risk assessment, and help establish whether or not a linear default is the appropriate extrapolation from the lowest cancer effect level. The 1996 US EPA *Proposed Guidelines for Carcinogen Risk Assessment* allow for a narrative risk characterization that incorporates mode of action.

What is known about the genotoxicity of arsenic with regard to profile and mechanism? Arsenic is unusual, although not unique, in that it does not appear to induce point mutations in standard bacterial assays, or to interact directly with DNA, but it does induce chromosomal alterations (aberrations and sister chromatid exchanges) both *in vitro* and *in vivo*. The profile is not clean, with not all studies reporting positive clastogenicity responses, especially for sister chromatid exchanges. For chromosome aberrations, the dose response curves are generally nonlinear, that could result either from two DNA lesions (directly or indirectly produced by arsenic) being necessary for a majority of the aberrations, or from an effect upon DNA repair.

It is difficult to explain a complete absence of point mutations since, in general, for the majority of chemicals, chromosome alterations and point mutations result from errors of DNA replication on a damaged template. The mutagenicity profile could be indicative of an effect on DNA repair that leads to misrepair events in the form of aberrations only. In this case, it would be predicted that chromosome-type aberrations, involving both chromatids as observed at metaphase, would be produced in cells in G_1. The data generally report the observation of chromatid-type aberrations, typically produced in G_2 or S, by radiation or radiomimetic chemicals. The human lymphocyte data show that chromatid-type aberrations are produced by arsenic exposure even in G_0/G_1 cells. If arsenic is indeed affecting DNA repair processes leading to a higher proportion of DNA damage in the S-phase, then the absence of point mutations is somewhat surprising, although an exhaustive set of studies on the induction of point mutations in humans and laboratory animals exposed *in vivo* has not been conducted. At this point, mode of action, namely a clear preference for the induction of chromosome aberrations, provides quite compelling evidence for developing nonlinear dose response models for predicting cancer risk at low exposures.

Toby Rossman

Overview on arsenic carcinogenesis

Direct genotoxicity of arsenic:

Arsenite (the most likely carcinogen) does not cause significant gene mutations at biologically relevant concentrations. The small amount of mutagenesis induced (at high dose) may be mainly deletions. It does cause various chromosomal effects (both structural and numerical) as well as gene amplification. Some of these effects (including deletions) may be due to oxidant stress, as arsenite can deplete glutathione.

The metabolite dimethlyarsinic acid (DMAA) can produce a peroxyradical which can damage DNA. The concentrations required for this effect may not be biologically relevant, and cannot explain the effects of arsenite in cells which do not methylate.

Indirect genotoxic effects:

Arsenite can enhance mutagenesis by other agents of various types. While it appears to inhibit DNA repair in cells, such inhibition is probably indirect, since no DNA repair enzyme has been found to be sensitive to arsenite inhibition in vitro. One candidate mechanism might be that arsenite blocks the p53-dependent DNA damage response, possibly by causing hypermethylation of the p53 promoter (i.e. loss of p53 expression). This would block DNA repair.

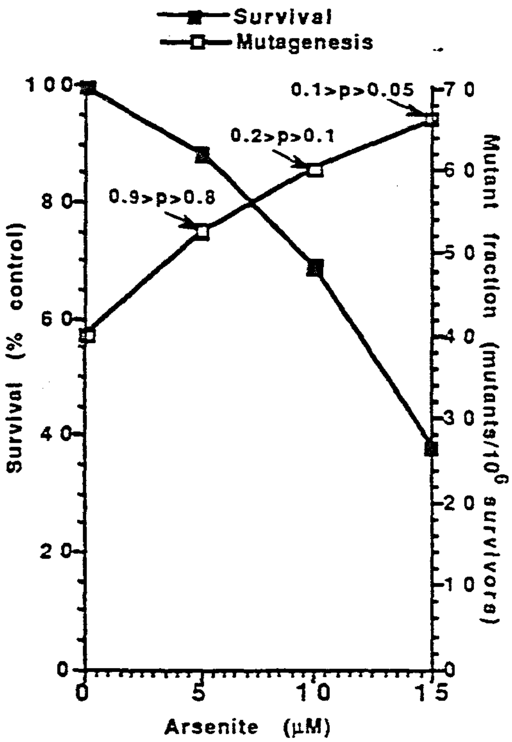
Carcinogenesis and risk assessment:

Although sometimes called a promoter, there is no good evidence that arsenite promotes. Rather, it should be considered to be a cocarcinogen. It is not clear how to model dose/response curves of a cocarcinogen at this time. Animal experiments should be attempted using long term low dose exposures to arsenite prior to the addition of a genotoxic insult (e.g. UVB) small enough to give a minimal effect alone. The shape of the dose/response curve could be obtained. However, it is possible that rodent experiments may not be suitable due to the acquisition of inducible tolerance to arsenite (lacking in human cells). It must be kept in mind that human cells are far more sensitive to arsenite compared with rodent cells.

On possible mechanisms of arsenic carcinogenesis

Arsenite (the most likely carcinogenic form of arsenic) is not generally mutagenic at single gene loci, and the very small numbers of induced mutants usually arise after exposure to highly toxic concentrations. The figure below illustrates the gene mutations induced by arsenite at the *E coli gpt* locus in an extremely sensitive transgenic cell line (data from Li and Rossman, 1989a). Given the very weak mutagenic activity (significance is not reached until ~40% of the cells are killed), arsenite is sometimes (mistakenly) considered a "non-genotoxic carcinogen". Partly because of this, the assumption is sometimes made that arsenite is a tumor promoter. There is little evidence for this view, as negative results were obtained in a bioassay testing for promotional activity (Milner, 1969). The metabolite dimethlyarsinic acid (DMAA) did (Yamamoto et al., 1995), but the significance of this finding for human exposure is questionable (see below).

Mutagenicity of arsenite
at the transgenic gpt locus in G12 cells



When Meng and Hsie (1996) analyzed the mutants resulting from another transgenic cell line treated with high concentrations of arsenite (which still gave mutant fractions only 2X background levels), the proportion of deletions was higher than in the spontaneous class. At more relevant concentrations, arsenite induces chromosome aberrations, aneuploidy, and micronuclei (reviewed in Rossman, 1994, 1997). Micronuclei (a marker of chromosome damage) are found in the bone marrow of mice treated with arsenite (Tinwell et al, 1991) and in exfoliated bladder cells from exposed humans (Warner et al., 1994).

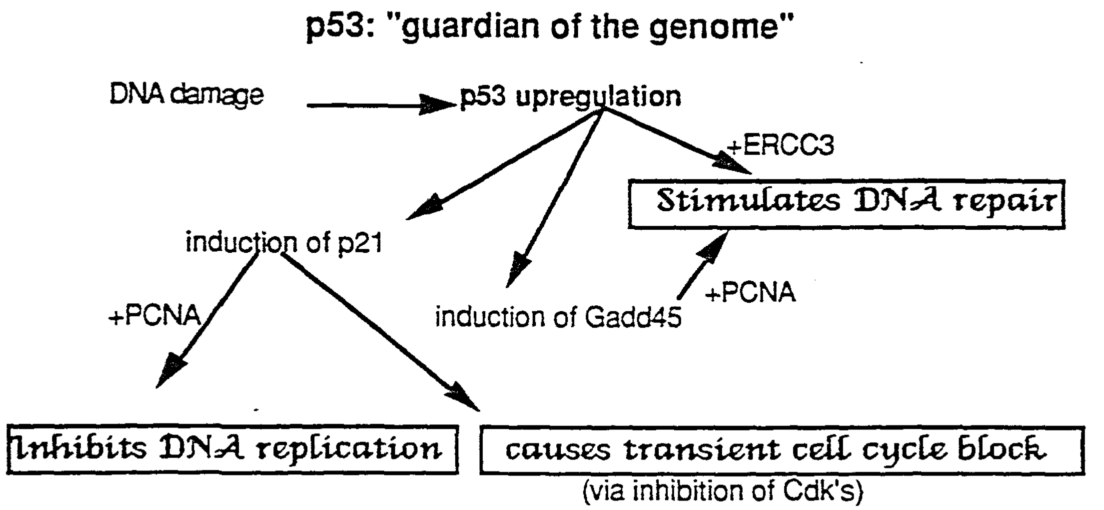
In humans, arsenic compounds are detoxified by methylation in the liver (reviewed in Aposhian, 1997) followed by excretion in the urine. Methylated metabolites are less toxic than arsenite or arsenate (Marafante et al., 1987). However, DMAA caused oxidative damage and DNA strand breaks in the mouse lung as well as in cultured cells. The strand breaks are apparently caused by the DMAA peroxy radical $(CH_3)_2AsOO\cdot$ (Yamanaka and Okada, 1994). It was suggested that this might explain how arsenite induces lipid peroxidation in various rat tissues (Ramos et al., 1995). However, doubts have been expressed that humans exposed to inorganic arsenic could accumulate sufficient DMAA (or its peroxy radical) for genotoxic effects to ensue. In normal human fibroblasts, the potency for clastogenicity is: arsenite>arsenate>DMAA (Oya-Ohta et al., 1996). In fact, >7 mM DMAA is required, whereas only 0.8 μM arsenite was clastogenic. Since the LD_{50}'s for arsenite in human cells range from about 0.2-2.0 μM (Rossman et al., 1997), concentrations in the mM range cannot be biologically meaningful for genotoxic effects in human cells. Nevertheless it is important to test the possibility that some of arsenite's genotoxic effects in human cells might be caused by DMAA.

Oxidative effects of arsenite may also be caused by caused by glutathione (GSH) depletion. Arsenite readily reacts with GSH, and GSH is required for reduction of arsenate to arsenite and in the reductive methylation of arsenite to DMAA (Scott et al., 1993). In the case of fibroblasts and other cells that do not appear to methylate arsenic and thus cannot generate DMAA peroxy radical, the most likely involvement of oxidant stress would be via depletion of GSH after arsenite treatment. Arsenite as well as DMAA (which is even more potent) can inhibit GSH reductase (Styblo et al., 1997), which would also lead to oxidant stress in the cell.

The concept that arsenite induces oxidative stress is supported by a number of other findings: 1) The addition of superoxide dismutase to the culture medium blocked arsenite-induced genotoxicity in human lymphocytes (Nordenson and Beckman, 1991). 2) Vitamin E (α-tocopherol) protects human fibroblasts from arsenite toxicity (Lee and Ho, 1994). 3) An x-ray sensitive, catalase deficient CHO cell variant is hypersensitive to killing and micronucleus induction by arsenite. Micronucleus induction was blocked by catalase (Wang and Huang, 1994). 4) Arsenite induces proteins which are induced by and protect against oxidative stress. These include metallothionein (MT) (Albores et al., 1992) and heme oxygenase (Keyse and Tyrrell, 1989), whose induction is blocked by antioxidants. We have shown that MT expression gives some protection against arsenite toxicity (even though MT doesn't have a high affinity for arsenite) (Goncharova et al., 1995). 5) Depletion of GSH increased the toxic and clastogenic effects of arsenite (Oya-Ohta et al., 1996).

Arsenite has been shown to enhance the mutagenicity and/or clastogenicity of many agents (reviewed in Rossman, 1994, 1997). Arsenite inhibits the completion of DNA excision repair, (Li and Rossman 1989a) probably via effects on DNA ligation (Li and Rossman 1989b; Lee-Chen et al, 1994). However, neither DNA ligases nor DNA polymerase α or β can be inhibited by arsenite concentrations many fold higher than those which can inhibit DNA repair in cells (Li, 1989; Li and Rossman, 1989b; E. Snow, personal communication). Arsenic also appears to act synergistically in lung carcinogenesis, e.g. with tobacco use in occupationally exposed workers (Hertz-Picciotto et al., 1992; Choiu et al., 1995) and with radon gas in tin miners (Xuan et al., 1993).

Arsenite induced gene amplification at the *dhfr* locus in SV40-transformed human keratinocytes cells, but failed to cause amplification of SV40 sequences (Rossman and Wolosin, 1992). This suggests that arsenite does not induce signaling typical of DNA-damaging agents (which induce SV40 amplification in this system), but rather affects checkpoint pathways such as those involving p53, whose disruption lead to cellular gene amplification (Livingstone et al., 1992). In fact, it is quite possible that arsenite blocks DNA repair by interfering with cell cycle checkpoints rather than by inhibiting repair enzymes. The tumor suppressor p53 has a crucial role as "guardian of the genome" in the control of cell cycle progression. If damaged DNA is replicated, it



may be mutated or lost due to chromosome breaks. DNA damage results in an accumulation of p53 protein, mainly via post-translational stabilization (Levine and Momand, 1990). p53 protein temporarily halts cell cycle progress, allowing time for DNA repair before replication (Kastan et al., 1991) or else causes apoptosis in heavily damaged cells (Miyashita et al., 1994). Cells with mutant p53 are more likely to continue to divide, and fail to undergo apoptosis, in spite of DNA damage to their chromosomes (Little, 1994). Such cells also show greatly elevated rates of chromosome aberrations such as deletions, translocations, amplifications and aneuploidy (Reznikoff et al., 1994; Hainaut, 1995), exactly the classes of genotoxic events induced by arsenite. p53 protein also plays a more direct role in DNA repair. Li-Fraumeni cells, which are p53-deficient, show reduced excision repair of pyrimidine dimers (Smith et al., 1995). As a transcription factor, p53 causes induction of Gadd45 and ERCC3 which, along with proliferating-cell nuclear antigen (PCNA), stimulate excision repair (See Figure above). p53 also induces p21$^{Waf\text{-}1/cip\text{-}1}$, a protein which binds to and inhibits cyclin-dependent protein kinases (Cdk's) and PCNA), resulting in G1 arrest and blockage of DNA replication. It is possible that arsenite blocks excision repair by interfering with p53 expression or activity. Mass and Wang (1997) have shown that long-term exposure of cells to low concentrations of arsenite resulted in hypermethylation of the p53 promoter, which is expected to result in blockage of p53 transcription. Cells with such a blockage would behave as p53 mutants (i.e. as Li-Faumeni phenocopies).

When p53 activity is inactivated by expression of the E6 protein of HPV16 in human cells, UV-induced mutations are elevated about 2-fold and a large increase in deletions is seen (Havre et al., 1995; Yu et al., in press), suggesting that deletion-prone intermediates, such as strand breaks or gaps, accumulate during faulty repair. Arsenite also increases UV-mutagenesis about 2-fold (Li and Rossman, 1991) and causes increased accumulation of strand breaks or gaps in cells with DNA damage (Li and Rossman, 1989a) suggesting a mechanism similar to that seen in cells with mutant or inactivated p53. Spontaneous gene amplification is rare in normal cells, but common in tumor cells which have mutated p53 genes (Livingstone et al., 1992). Double strand breaks have been implicated as a possible cellular signal for gene amplification (Nelson and Kastan, 1994). Arsenite causes gene amplification in SV40-transformed human keratinocytes (Rossman and Wolosin, 1992). Although human keratinocytes are a highly relevant system in which to study arsenite, the SV40 T-antigen inactivates the p53 protein in these cells, allowing gene amplification. It is not known whether long term exposure to arsenite would induce gene amplification in human cells with normal p53.

No studies have been carried out identifying changes in oncogenes or tumor suppressor genes in arsenic-induced skin cancers. Bladder tumors from Taiwanese who had high levels of arsenic in their drinking water showed p53 mutations (62%), mostly transitions (Shibata et al., 1994). The most notable feature of these mutations were the existence of double mutations in 3 of the 8 tumors which had mutations. This is normally an extremely rare event, and suggests genomic instability as a cause, as well as a consequence, of these mutations. The genotoxic effects of arsenite <u>alone</u> might be more likely to result in loss of tumor suppressor functions (e.g. by deletion or silencing) than in mutation. However, by blocking DNA repair, arsenite would also enhance mutagenesis of a second (mutagenic) agent.

One of the unexplained facts about arsenic carcinogenesis is the difficulty in finding a good animal model, since most attempts to induce tumors by arsenic compounds in rodents have failed. This might be related to inappropriate dosage or treatment regimens. Arsenite should be tested as a co-carcinogen. Arsenite-induced genomic instability might develop gradually. Thus, long term arsenite treatment (in the drinking water) might be necessary before a significant fraction of cells are affected, and a second genotoxic carcinogen should then be used. On the other hand, it is possible that, because rodents cells may have an arsenite-inducible tolerance mechanism not seen in human cells (Rossman et al., 1997), all rodent carcinogenicity experiments (except those using extremely toxic, short term exposure) might be doomed to failure. But it's worth a try.

Albores, A., Koropatnick, J., Cherian, M.G., and Zelazowski, A.J. Arsenic induces and enhances rat hepatic metallothionein production in vivo. Chem-Biol. Interact. 85:127-140, 1992.

Aposhian, H.V. Enzymatic methylation of arsenic species and other new approaches to arsenic toxicity. Ann. Rev. Pharmacol. Toxicol. 1997 in press.

Choiu, H.-Y., Hsueh, Y.-M., Liaw, S.-F., Chiang, M.-H. et al. Incidence of internal cancers and ingested inorganic arsenic: A seven-year follow-up study in Taiwan. Cancer Res. 55:1296-1300, 1995.

Goncharova, E.I. and Rossman, T.G. The antimutagenic effects of metallothionein may involve free radical scavenging. in: *Genetic Response to Metals.* (B. Sarkar, Ed.) Marcel Dekker, Inc., New York pp. 87-100, 1995.

Hainaut, P. The tumor suppressor protein p53: a receptor to genotoxic stress that controls cell growth and survival. Current Opinion in Oncology 7:76-82, 1995.

Havre, P.A., Yuan, J., Hedrick, L., Cho, K.R., and Glazer, P.M. p53 inactivation by HPV16 E6 results in increased mutagenesis in human cells. Cancer Res. 55:4420-4424, 1995.

Hertz-Picciotto, I., Smith, A.H., Holtzman, D., Lipsett, M., and Alexeeff, G. Synergism between occupational arsenic exposure and smoking in the induction of lung cancer. Epidemiology 3:23-31, 1992.

Kastan, M.B., Onyekwere, O., Sidransky, D., Vogelstein, B., and Craig, R.W. Participation of p53 protein in the cellular response to DNA damage. Cancer Res. 51:6304-6311, 1991.

Keyse,. S.M., Tyrrell, R.M. Heme oxygenase is the major 32-kDa stress protein induced in human skin fibroblasts by UVA radiation, hydrogen peroxide, and sodium arsenite. Proc. Natl. Acad. Sci. USA 85:99-103, 1989.

Lee, T.C. and Ho, I.C. Differential cytotoxic effects of arsenic on human and animal cells. Environ. Health Perspect. 3:101-105, 1994.

Lee-Chen, S.F., Yu, C.T., Wu, D.R., and Jan, K.Y. Differential effects of luminol, nickel, and arsenite on the rejoining of ultraviolet light and alkylation-induced DNA breaks. Environ. Mol. Mutagenesis 23:116-120, 1994.

Levine, A.J., and Momand, J. Tumor suppressor genes: the p53 and retinoblastoma sensitivity genes and gene products, Biochim. Biophys. Acta. 1032:119-136, 1990.

Li, J.-H. and Rossman, T.G. Comutagenesis of sodium arsenite with ultraviolet radiation in Chinese hamster V79 cells. Biol. Metals 4:197-200, 1991.

Li, J.-H. Ph.D. Thesis, New York University, 1989.

Li, J.-H. and Rossman, T.G. Mechanism of comutagenesis of sodium arsenite with N-methyl-N-nitrosourea. Biol. Trace Elem. Res. 21:373-381, 1989a.

Li, J.-H., and Rossman, T.G. Inhibition of DNA ligase activity by arsenite: A possible mechanism of its comutagenesis. Molec. Toxicol. 2:1-9, 1989b.

Little, J.B., Failla Memorial Lecture: changing views of radiosensitivity. Radiat. Res. 140:299-236, 1994.

Livingstone, L.R., White, A., Sprouse, J., Livanos, E., Jacks, T., and Tlsty, T.D. Altered cell cycle arrest and gene amplification potential accompany loss of wild-type p53. Cell 70:923-935, 1992.

Marafante, E., Vahter, M., Norin, H., Envall, J., Sandstrom, M., Christakopoulos, A., and Ryhage, R. Biotransformation of dimethylarsinic acid in mouse, hamster and man. J. Appl. Toxicol. 7:111-117, 1987.

Mass, M.J., and Wang, L. Arsenic alters cytosine methylation patterns of the promoter of the tumor suppressor gene p53 in human lung cells: A model for a mechanism of carcinogenesis. Mutat. Res. 1997, in press.

Meng, Z., and Hsie, A.W. Polymerase chain reaction-based deletion analysis of spontaneous and arsenite-enhanced gpt mutants in CHO-AS52 cells. Mutat. Res. 356:255-0259, 1996.

Milner, J.E. The effects of ingested arsenic exposure on methyl cholanthrene induced skin tumours in mice. Arch. Environ. Health 18:7-11, 1969.

Miyashita, T., Krajewski, S., Krajewska, M., Wang, H.G., Lin, H.K., Liebermann, D.A., Hoffman, B., and Reed, J.C. Tumor suppression p53 is a regulator of bcl-2 and bax gene expression in vitro and in vivo. Oncogene 9:1799-1805, 1994.

Nelson, W.G., and Kastan, M.B. DNA strand breaks: the DNA template alterations that trigger p53-dependent DNA damage response pathways. Mol. Cell. Biol. 14:1815-1823, 1994.

Nordenson, I. and Beckman, L. Is the genotoxic effect of arsenic mediated by oxygen free radicals? Hum. Hered. 41:71-73, 1992, 1991.

Oya-Ohta, Y., Kaise, T., Ochi, T. Induction of chromosomal aberrations in cultured human fibroblasts by inorganic and organic arsenic compounds and the different roles of glutathione in such induction, Mutat. Res. 357:123-129, 1996.

Ramos, O., Carrizales, L., Yanez, L., Mejia, J., Batres, L. Ortiz, D., and Diaz-Barriga, F. Arsenic increased lipid peroxidation in rat tissues by a mechanism independent of glutathione levels. Environ. Health Perspect. 103:85-88, 1995.

Reznikoff, C.A., Belair, C., Savelieva, E., Zhai, Y., Pfeifer, K., Yeager, T., Thompson, K.J., DeVries, S., Bindley, C., and Newton, M.A. Long-term gnome stability and minimal genotypic and phenotypic alterations in HPV16 E7-, but not E6-, immortalized human urepithelial cells. Genes Dev. 8:2227-2240, 1994.

Rossman, T.G. and Wolosin, D. Differential susceptibility to carcinogen-induced amplification of SV40 and *dhfr* sequences in SV40-transformed human keratinocytes. Mol. Carcinogen. 6:203-213, 1992.

Rossman, T.G. Metal mutagenesis. In: Goyer, R.A., Cherian, M.G., eds. Toxicology of Metals- Biochemical Aspects. New York: Springer-Verlag, 374-405, 1994.

Rossman, T.G., Goncharvoa, E.I. Nádas, A. and Dolzhanskaya, N. Chinese hamster cells expressing a metallothionein antisense sequence become spontaneous mutators. Mutat. Res. 373:75-85, 1997.

Scott, N., Hatlelid, K.M., MacKenzie, N.E., and Carter, D.E. Reactions of arsenic (III) and arsenic (V) species with glutathione. Chem. Res. Toxicol. 6:102-106, 1993.

Shibata, A., Ohneseit, P.F., Tsai, Y.C., Spruck C.H. III, Nichols, P.W., Chiang, H.-S., Lai, M.-K., and Jones, P.A. Mutational spectrum in the p53 gene in bladder tumors from the endemic area of black, foot disease in Taiwan. Carcinogenesis 15:1085-1087, 1994.

Smith, M.L., Chen, I., Zhan, Q., O'Connor, P.M., and Fornace, A.J. Involvement of p53 tumor suppressor in repair of UV-type DNA damage. Oncogene 10:1053-1059, 1995.

Styblo, M., Serves, S.V., Cullen, W.R., and Thomas, D.J. Comparative inhibition of yeast glutathione reductase by arsenicals and arsenothiols. Chem. Res. Toxicol. 10:27-33, 1997.

Tinwell, H., Stephens, S.C., and Ashby, J. Arsenite as the probable active species in the human carcinogenicity of arsenic: mouse micronucleus assays on Na and K arsenite, orpiment, and Fowler's solution. Environ. Health Perspect. 95:205-210, 1991.

Wang, T.S. and Huang, H . Active oxygen species are involved in the induction of micronuclei by arsenite in XRS-5 cells. Mutagenesis 9:253-257, 1994.

Xuan, X.Z., Lubin, J.H., Li, J.Y., Yang, L.F., Luo, A.S., Lan, Y., Wang, J.Z., and Blot, W.J. A cohort study in southern China of tin miners exposed to radon and radon decay products, Health Physics 64L:120-131, 1993.

Yamamoto, S. , Y. Konishi, T. Matsuda, T. Murai, M. Shibata, I. Matsui-Yuasa, S. Otani, K. Kuroda, G. Endo, and S. Fukushima . Cancer induction by an organic arsenic compound, dimethylarsinic acid (cacodylic acid), in F344/DuCrj rats after pretreatment with five carcinogens. Cancer Res. 55:1271-1276, 1995.

Yamanaka, K. and Okada, S. Induction of lung-specific DNA damage by metabolically methylated arsenics via the production of free radicals. Environ. Health Perspect. 102(3):37-40, 1994.

Yu, Y., Li,C.-Y., Little, J.B. Abrogation of p53 function by HPV16 E6 gene delays apoptosis and enhances mutagenesis but does not alter radiosensitivity in K6 human lymphoblast cells. Oncogene 1997, in press.

Appendix D

AGENDA

United States
Environmental Protection Agency
National Center for Environmental Assessment

Expert Panel on Arsenic Carcinogenicity

Holiday Inn—National Airport
Washington, DC
May 21-22, 1997

Agenda

WEDNESDAY, MAY 21, 1997

8:00AM Registration

9:00AM **Chair's Opening Remarks and Review of Premeeting Comments** .... R. Julian Preston, Chair
Chemical Industry Institute of Toxicology
Research Triangle Park, NC

9:10AM **The Proposed 1996 Safe Drinking Water Act Charge on Arsenic** .... James Taft
Office of Water
U.S. Environmental Protection Agency (U.S. EPA)
Washington, DC

9:20AM **Introduction/Background** .... Jeanette Wiltse
Director, Health and Ecological Criteria Division
Office of Science and Technology, Office of Water
U.S. EPA
Washington, DC

9:30AM **Chair's Summary of Panelists' Comments** .... R. Julian Preston

9:50AM BREAK

10:05AM **Fundamentals of Carcinogenesis** .... Samuel Cohen
University of Nebraska Medical Center
Omaha, NE

10:20AM **Charge to Panel** .... R. Julian Preston

10:30AM **Issue No. I: What Do the Existing Data Tell Us About Arsenic's Carcinogenic Mode of Action (MoA)?**
- ❑ How does arsenic affect DNA?
- ❑ Are there important determinants other than effects on DNA (e.g., tissue injury)?

WEDNESDAY, MAY 21, 1997 (continued)

11:45AM Wrap-Up

12:00PM LUNCH

1:00PM **Issues No. I (continued)**
- ❑ Is Arsenic carcinogenicity influenced by metabolism?
- ❑ What are the possible roles of metabolites?
- ❑ Does the tumor data (both human and animal studies) give clues as to arsenic's MoA?

3:00PM BREAK

3:15PM **Issue No. 2: What Is the Level of Confidence About Conclusions Regarding Arsenic's MoA?**
- ❑ What uncertanties exist on the MoA?
- ❑ Are there alternative hypotheses?
- ❑ Does the body of evidence fit with a generally accepted MoA (i.e., is there consistency, depth, breath, concordance, consensus)?
- ❑ Is the MoA consistent with generally agreed upon theories of carcinogenesis?

4:15PM **Observer Comments**
- ❑ Observers must sign up at registration desk before the comment period

4:45PM **Closing Comments** . . . . R. Julian Preston

5:00PM ADJOURN

THURSDAY, MAY 22, 1997

9:00AM **Issue No. 3: What Are the Dose-Response Implications of the MoA Understanding?**
- ❑ Is the MoA information consistent with a low-dose linear extrapolation approach, a nonlinear procedure, or with both procedures?
- ❑ Do the data on precursor events underlying tumor effects provide information on the shape of the dose-response relationship for tumor induction?

10:30AM BREAK

10:45AM **Summary of Issues and Recommendations** . . . . R. Julian Preston

12:00PM Wrap-Up

12:15PM ADJOURN

1:30PM **Writing Session** (Panel Members Only)
Workshop Summary Report

4:00PM Writing Session Ends